U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-275 978

Journal Articles on Pesticide Chemical Analysis Group 3

Environmental Protection Agency, Washington, D C

1969

Mother Sheet

PB 275 978

Title:

Journal Articles on Pesticide Chemical Analysis. Group 3.

Improved Amino Acid Analysis
II. Automated Integration and Computer Processing of Data

Verbesserte Aminosäure-Analyse
II. Automatisierte Integration und Computer-Auswertung der Daten

Analyse améliorée d'amino-acides
II. Intégration automatisée et traitement des données par un ordinateur

PB 275 979
PUBLICATION
334
PESTICIDES PROGRAM
National Communicable
Disease Center
Atlanta, Ga. 30333

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New methodologies for the rapid determination of amino acids by ion-exchange chromatography have presented the highly instrumented laboratory with a vast volume of data to be calculated. Various manual methods of calculation (height-width, absorbence or planimetry) are time consuming and cannot be used to maintain an efficient balance between increased production of information and finished data. Electronic digital integrators have helped to eliminate manual interpretation from the strip chart recordings, but manual calculation must still be performed to obtain the final data. The ideal system would eliminate all manual handling of the data [1, 2]. The following describes such a system used by this laboratory in conjunction with Spinco 120 C [1]) amino acid analyzer.

The Infotronics [1]) CRS-40/50 system contains the following components for amino acid analysis data processing:

1. Multiple channel tape recorder.
2. Tape playback.
3. CRS-100T Integrator.
4. CRS-50 Data Processor.
5. Teletype Printer.

A three channel tape recorder monitors the analog signal from each of the colorimeters. The visual strip chart recording is also available. The analog signal is converted by the tape recorder to a digital signal which is recorded on magnetic tape. The tape recorded chromatogram is then placed on a tape playback unit, which monitors both a reference signal associated with baseline and the signal on the selected sample channel. The integrator senses changes in frequency and integrates the signal according to specifications given by the operator. These specifications are optimized by the proper setting of the baseline tracking rates, slope sensitivity, trip level and filter frequency. Table 1 presents the data as seen by the data processor from the integrator. It contains

TABLE 1

RETENTION TIME AND AREA DATA FPOM INTEGRATOR

Ø11Ø378100[1]

TIME	AREA
69	834
1Ø3	4693
195	25909
363	2Ø45Ø
422	469511
453	445338
49Ø	467988
713	461631
83Ø	455852
886	459369
147	482Ø54
237	246435
312	13413
352	393225
391	51Ø1Ø6
424	511449
454	523707
585	49Ø26Ø
63Ø	493407

[1] Identification

an identification code which denotes the source of the analysis (011), analysis number (0378), the dilution factor (100), the retention time, and the peak area. Non-printing commands, begin event and reset, are also supplied by the integrator.

The program supplied with the data processor performs the following operations:

1. Entry of identification information, analysis constants, parameters, etc.
2. Data acquisition and storage on a real time basis.
3. Data processing and analysis upon completion of the tape playback.
   a) Correction of retention time from 3 to 4 digits.
   b) Selection of reference peak(s) according to specifications given in the data table.
   c) Peak identification by use of retention time data.
   d) Area correction.
   e) Quantitative analysis.
   f) Report printout.

The quantitative analysis may be one of seven analytical procedures:

(1) Percent composition without component identification or correction factor;
(2) Percent composition with component identification using absolute time;

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(3) Percent composition with component identification using relative time;
(4) Absolute composition with component identification using absolute time;
(5) Absolute composition with component identification using relative time;
(6) Internal standard with component identification using absolute time;
(7) Internal standard with component identification using relative time.

The tabulated results from the data processor in Table 2 reports the input as described in Table 1.

TABLE 2 DATA PROCESSOR OUTPUT

DATE
TIME

SAMPLE NO. Ø378
SAMPLE TYPE Ø1 AA ANAL SD

ANALYZER Ø1
ANALYSIS TYPE 7
INPUT CHANNEL 11
DILUTION FACTOR 1.ØØØ
REL. TIME CORR. .999

AREA	TIME	CONC	CONSTITUENT
834	69	.031	UNKNOWN
4693	1Ø3	.179	UNKNOWN
25ØØ9	195	.989	UNKNOWN
2Ø45Ø	363	.78Ø	UNKNOWN
469511	422	19.989	AS
445338	453	19.994	TH
467988	49Ø	19.995	SR
461631	712	2Ø.Ø21	G2
455852	829	19.979	G3
459369	885	19.993	AL
482Ø54	1146	2Ø.Ø81	VA
246435	1236	2Ø.155	C3
13413	1311	.512	UNKNOWN
393225	1351	2Ø.ØØØ	ME
51Ø1Ø6	139Ø	2Ø.ØØ3	IL
511449	1423	19.998	LU
523707	1453	19.999	SD
49Ø26Ø	1583	19.972	TY
493407	1628	19.971	ØA

The analysis type 7 was employed in this laboratory. The computer program is written so that five significant figures appear in the concentration (CONC) column. To obtain the final data in μ moles, the decimal is moved two places to the left. Relative time correction (REL. TIME CORR.) is a factor by which all the values for time are multiplied in order to correct all values relative to the retention time of the internal standard. The areas for each component are identical as seen in Tables 1 and 2. Time is expressed in units of 0.1 minute. The CONC column figures represent 0.200 μ mole/2 ml of standard amino acid calibration solution and internal standards, norleucine and α-amino-γ-guanidobutyric acid. A constituent is designated "UNKNOWN" when not accounted for in the data tables. The individual amino acids are abbreviated as follows: Aspartic Acid, AS; Threonine, TH; Serine, SR; Glutamic Acid, G2; Glycine, G3; Alanine, AL; Valine, VA; Cysteine, C3; Methionine, ME; Isoleucine, IL; Leucine, LU; Norleucine, SD; Tyrosine, TY; Phenylalanine, ϕA.

The accuracy of the computer analysis system is compared with the manual height-width method in Table 3. Proline is not shown in the table and will continue to be calculated by hand. Because of the separation problem and the relatively large concentration difference of valine and cysteine in our samples, these two amino acids will also be calculated by hand.

TABLE 3 COMPARISON OF MANUAL CALCULATION WITH COMPUTER CALCULATION

μmoles ± S.E.

AMINO ACID	MANUAL CALCULATION	COMPUTER CALCULATION
ASPARTIC	0.1989 ± 0.0097	0.2019 ± 0.008
THREONINE	0.1991 ± 0.0051	0.1999 ± 0.003
SERINE	0.1993 ± 0.0038	0.1996 ± 0.003
GLUTAMIC	0.1990 ± 0.0020	0.2019 ± 0.004
GLYCINE	0.2002 ± 0.0061	0.2009 ± 0.006
ALANINE	0.1995 ± 0.0026	0.1953 ± 0.001
VALINE	0.1997 ± 0.0041	0.2147 ± 0.009
HALF CYSTINE	0.1998 ± 0.0090	0.1980 ± 0.005
METHIONINE	0.1996 ± 0.0021	0.2002 ± 0.003
ISOLEUCINE	0.1981 ± 0.0050	0.1997 ± 0.001
LEUCINE	0.1987 ± <0.001	0.2000 ± <0.001
NORLEUCINE*	0.2000 ± <0.001	0.2000 ± <0.001
TYROSINE	0.1996 ± 0.0018	0.1996 ± 0.003
PHENYLALANINE	0.1994 ± 0.0042	0.1976 ± 0.001
LYSINE	0.2007 ± 0.0060	0.1990 ± 0.006
HISTIDINE	0.1995 ± 0.0064	0.1983 ± 0.007
ARGININE	0.2002 ± 0.0010	0.2002 ± 0.002
α-AMINO γ-GUANIDOBUTYRIC*	0.2000 ± <0.001	0.2000 ± <0.001

* Internal Standards

It is readily seen in Table 3 that excellent agreement between the two methods is apparent. The length of time required to obtain the data by computer is limited by rate of playback of the magnetic tape. In our particular system, the playback time is 15 minutes. The system could be further automated by using on-line integrators, thus bypassing the tape playback, however, expanded memory would be required. Further work in our laboratory will assess the relative accuracy of the two systems at lower levels of amino acids from in vivo sources.

The authors are grateful for the assistance of Mr. *John Heinz*, Infotronics Corporation, Houston, Texas, USA.

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Received May 3, 1969
Accepted May 15, 1969

CHROMATOGRAPHIA, 1(11-12):406-409,
NOVEMBER-DECEMBER 1968.

PB 275 980

An improved Method of Automation of Amino Acid Analysis

Ein verbessertes Verfahren zur Automation der Aminosäure-Analyse

Une méthode améliorée pour l'automation de l'analyse des amino-acides

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Amino acid analysis of physiological fluids with a Beckman [1] Model 120C amino acid analyzer requires the use of 2 columns and 2 buffers for each column. A standard analysis requires a long column (0.9 x 69 cm) for the separation of acidic and neutral amino acids by 2 buffers, A and B, pH 3.20, 0.2 M Na^+ and pH 4.26, 0.2 M Na^+, respectively. The basic amino acids are separated on a short column (0.9 x 29 cm) by buffers C and D, pH 4.25, 0.38 M Na^+ and pH 5.36, 0.35 M Na^+, respectively [1]. The discrete buffer change at a preset time from A to B in the long column is automatic. Buffers A and B may also be switched manually when desired. However, only manual switching is provided for buffers C and D. One method for automating the short column involves mounting the slide valve block II for buffers C and D to the slide valve block I for buffers A and B, which is an integral part of the pre-existing Motovalve assembly [2]. The lever arms of slide valve blocks I and II are linked together with a cotter pin, and the pin is connected to the movable arm of the Motovalve cam. Since the lever arms are linked together, only 2 buffer arrangements can be obtained:

block I, buffer A and block II, buffer C.
block I, buffer B and block II, buffer D.

In our laboratory, a short column analysis is started in the morning, while the long column, which was used the previous day, is being regenerated with NaOH under nitrogen pressure. The regeneration of the long column requires a maximum of 4 hr. The equilibration of the long column with buffer A under nitrogen pressure would require a minimum of 4 hr., whereas equilibration by pumping requires only 1 hr. Buffer A cannot be pumped through the long column because the buffer change for the short column took place 185 min. after the analysis was started.

At this point block I contains buffer B and block II contains buffer D. It is not possible to equilibrate the long column with buffer A while the short column is in operation with its second buffer. It can be seen that the following additional block-buffer combinations are required to obtain full benefit from automating the short column:

block I, buffer A and block II, buffer D.
block I, buffer B and block II, buffer C.

The following describes an alternate means of automation, which has the capabilities described above. This modification requires the following parts [2]).

1 ea. No. 313373 Motovalve less part no. 313370 slide valve.
2 ea. No. 837998 Switch, maintained contact.
2 ea. No. 837996 Light indicator, white.
4 ea. No. 836927 Lamp, neon.
4 ea. No. 015070 Resistor, 68K, 1/2W, 5%.
2 ea. No. 837997 Cap.
10 ft. 306903 Teflon tubing, 1/8" O.D.

The method requires the insertion of 2 maintained contact switches between the power source and relay K1 and a duplication of the existing Motovalve circuit for the second Motovalve [3]. The maintained contact switches allow the selection of Motovalve I or II for the automatic buffer change mode or manual mode. Fig. 1 shows the wiring of these switches. Buffer switch 1 (BS1) has 2 wires to common (C): wire No. 18 which is the power line, and wire No. 16 which provides power to contact No. 3 of relay K2.

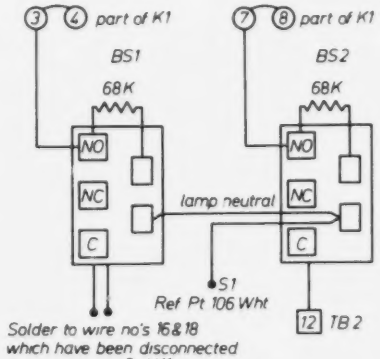

Fig. 1

- Wiring of Buffer Switches 1 and 2

NC - Normally Closed
NO - Normally Open
C - Common

- Schaltbild der Pufferschalter 1 und 2
- Câblage des commutateurs-tampon 1 et 2

[1]) Use of trade names is for identification purposes only and does not constitute endorsement by the U. S. Public Health Service.

[2]) Beckman Instruments, Inc., Spinco Division, Palo Alto, California.

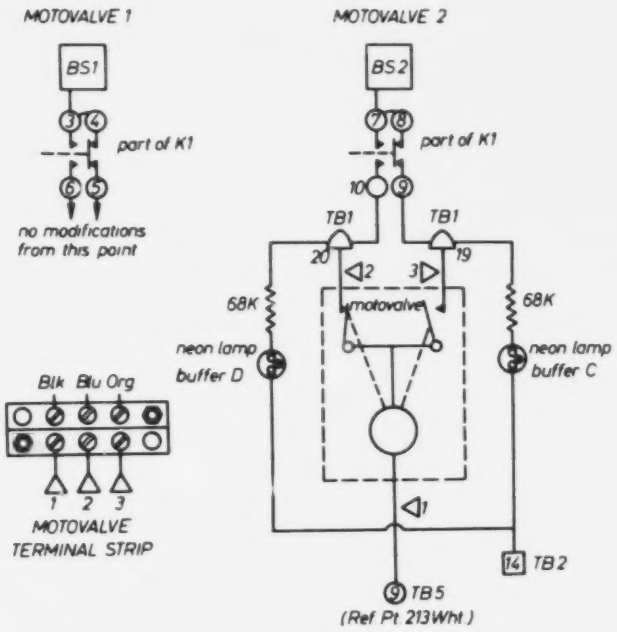

Fig. 2

- Motovalve II Schematic with its Terminal Strip Connections
- Schema des selbsttatigen Ventils II mit Klemmleisten-Anschlüssen
- Diagramme de la moto-vanne II avec ses connexions aux bornes

The current to buffer switch 2 (BS2) is supplied directly from terminal board 2, point 12. The neutral side of both buffer switch neon lamp circuits is grounded to switch (S1) at reference point, wire No. 106 white. The wires to and from BS1 and BS2 are 18 AWG, multistrand; except for the neon lamp ground, which is 20 AWG, multistrand. All other connections are also 20 AWG, multistrand [4]. The circuit for the additional Motovalve is shown in Fig. 2. The previously unused portion of K1 supplies relay switching for Motovalve II. Terminal board space is available on terminal board 1 (TB1). Terminal board 5 (TB5) is the barrier strip mounted behind the pump rack. The buffer change block for buffers C and D, which is provided with the analyzer, is used with Motovalve II. Motovalve II is mounted above Motovalve I. All switches and indicator lights are mounted on the front of the instrument panel for accessibility and visibility.

Automation of the short column allows the complete analysis of a sample in one working day without regard to which column is utilized first.

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Received July 29, 1968
Accepted Aug. 29, 1968

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PB 275 981

Nuclear Quadrupole Resonance Spectrometry of Some Chlorinated Pesticides

PUBLICATION PUBLICATION
297

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Chlorine nuclear quadrupole resonance (NQR) spectrometry has been employed to demonstrate the difference in the chemical environment of the ^{35}Cl atoms in several organochlorine compounds commonly used as pesticides. Correlations between resonance frequency and structure have been made in four isomers of 1,2,3,4,5,6-hexachlorocyclohexane (α-,β-,γ-,δ-HCH) and in certain cyclodiene chlorinated insecticides including 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*, *exo*-5,8-dimethanonaphthalene (aldrin), 1,2 3,4, 10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*, *exo*-5,8-dimethanonaphthalene (dieldrin), 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (heptachlor), 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene (heptachlor epoxide), 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*, *endo*-5,8-dimethanonaphthalene (endrin), and *cis*-1, 2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4, 7-methanoindene (α-chlordane). Assignment of signals to specific chlorine atoms has been made utilizing spectra-structure correlation charts and other data. Some of the problems encountered in the interpretation of NQR spectra are discussed.

PURE QUADRUPOLE RESONANCE studies are useful in the determination of the distributions of bonding electrons as well as steric distortions in compounds containing isotopes with nonzero quadrupole moments (*1*). Recently, Brame has reviewed the analytical aspects of nuclear quadrupole resonance with particular emphasis on the use of the technique for structure determination of compounds containing ^{14}N and ^{35}Cl nuclei (*2*). In an NQR experiment, radiation in the radio frequency region is employed to effect transitions among the various orientations of a quadrupolar nucleus in the asymmetric electric field of the bonding electrons. Structural information about a compound may be obtained by considering how different steric and electronic effects influence the asymmetry of the electronic environment and simultaneously the quadrupole coupling constant and the resonance frequency. In this manner, first order structure determinations can be attempted on the basis of peak positions. Additionally, peak areas may be used in interpreting the spectra of unknown compounds since the signal strength is proportional to the number of nuclei giving rise to the signal. This latter property has found recent application in the analysis of mixtures of organic and inorganic compounds (*3*). Attempts to utilize NQR frequency resonances in structure determinations of unknown compounds, however, while theoretically sound, have been rare and have met with only qualified success (*4*).

In this paper we apply the NQR technique to the determination of structure of the four isomers of 1,2,3,4,5,6-hexachlorocyclohexane and six cyclodiene chlorinated insecticides.

EXPERIMENTAL

Resonance frequency data reported in Figure 1 were obtained from two comprehensive literature sources (*1*, *5*). The chlorinated pesticides used in this work were analytical grade materials of 99% or better purity as determined by electron capture gas chromatography. The α-, β-, and δ-isomers of 1, 2, 3, 4, 5, 6-hexachlorocyclohexane were purchased from Pierce Chemical Co., Rockford, Ill. The remaining chlorinated pesticides were obtained without charge from the manufacturer through the Pesticides Repository of this laboratory. Individual sources of these materials are as follows: γ-1, 2, 3, 4, 5, 6-hexachlorocyclohexane, Hooker Chemical Co., Niagara Falls, N.Y.; aldrin, dieldrin, and endrin, Shell Chemical Co., New York, N.Y.; heptachlor, heptachlor epoxide, and α-chlordane, Velsicol Chemical Co., Chicago, Ill. In the case of aldrin, an absence of resonances was considered due to a lack of instrument sensitivity or considerable disorder in the crystal lattice. In an attempt to circumvent this latter possibility, the aging technique (*6*, *7*) was used in which a sample of aldrin was maintained for several days at a temperature just below the melting point. Aging produced no detectable effect on the NQR spectrum, however. In addition, samples of aldrin were crystallized from hexane, acetone, and benzene with no apparent change in the NQR spectrum.

The spectra were obtained using a Wilks Scientific Model NQR-1 nuclear quadrupole resonance spectrometer which is based on the design of Peterson and Bridenbaugh (*8*). One-gram samples of the chlorinated pesticides were placed in a 2-dram thin walled sample vial, positioned in the spectrometer coil, and the spectra were recorded at 22 ± 2° C. Identification and frequency measurements of the resonances were made by means of a James Millen Mfg. Co. type 90661 grid dip meter with a reference oscillator used for beating with the superregenerative oscillator. Frequency measurements are accurate to ± 0.1 MHz. Sweep speeds of approximately 0.5 to 1.0 MHz per hour were employed. The NQR data are reported in terms of resonance frequency rather than as the quadrupole coupling constant (eQq/h). In the case of ^{35}Cl, which has a spin quantum number of 3/2, the coupling constant can be obtained by multiplying the resonance frequency by 2.

RESULTS AND DISCUSSION

Accumulation of data indicates that spectra-structure correlations can be made utilizing NQR frequency resonances in

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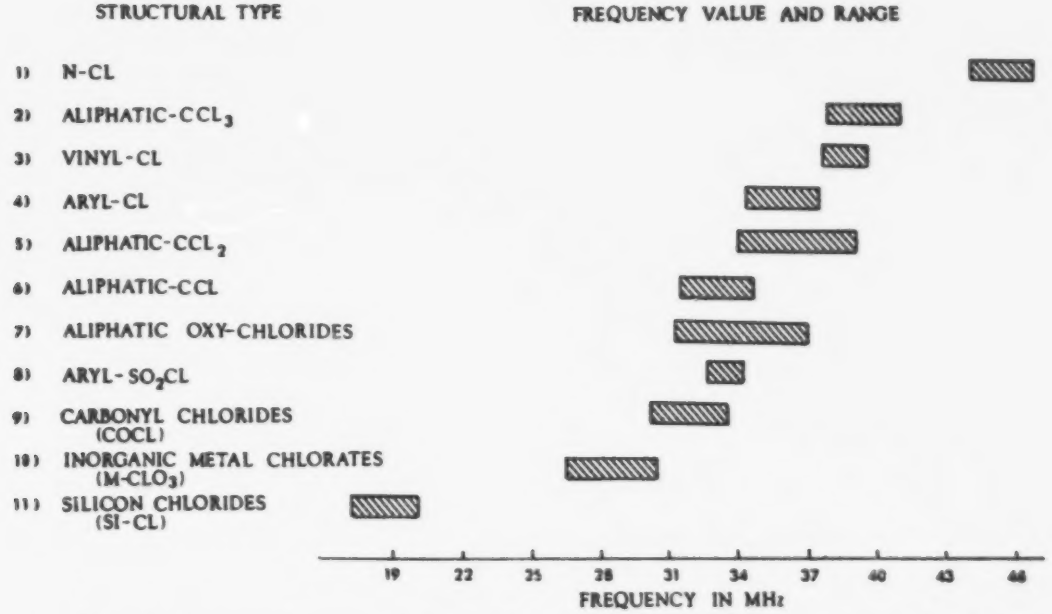

Figure 1. Resonance frequency–structure correlations of ^{35}Cl pure quadrupole resonances at 77 °K

much the same manner as is done in infrared and nuclear magnetic resonance spectrometry. Figure 1 illustrates a correlation chart of NQR frequencies for ^{35}Cl resonances in the range 18–46 MHz at 77 °K. The frequency range of covalent ^{35}Cl resonances in organic compounds is approximately 30–40 MHz. In general, the less negative a chlorine atom, the higher the frequency of absorption. Relatively unhindered aliphatic chlorine atoms (R—CCl) appear at lower frequency. Increasing the steric crowding in proceeding from R—CCl_2 to R—CCl_3 in this series results in a shift to higher frequency. This shift is more properly interpreted in terms of the changes in ionic character of the C—Cl bond. The ionic character of the C—Cl bond which is negative at the chlorine atom, decreases with an increasing number of chlorine substituents, and an increase in the resonance frequency is observed. This is best illustrated by the two classes of compounds in the extreme frequency ranges of Figure 1. Resonance signals of Si-Cl groups being most negative at the chlorine atom appear at the lower frequency range whereas signals of N-Cl groups being least negative at the chlorine atom appear at the higher frequency range.

The NQR spectra of the four isomers of 1,2,3,4,5,6-hexachlorocyclohexane have been reported (*9*) and assignments of the signals for the β- and γ-isomers have been made (*2*). The data obtained in this work for the four isomers are reported in Table I and compared with the literature values. The following resonance frequency assignments have been made for the β- and γ-isomers (*2*). The single signal observed

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Table I. ^{35}Cl Pure Quadrupole Resonances of Isomers of 1,2,3,4,5,6-Hexachlorocyclohexane (in MHz at Ambient Temp.)

Compound	Structure	Resonance frequency (this work)	Lit. value (*2*)	Lit. value (*9*)
1*e*,2*e*,3*e*,4*e*,5*e*,6*e*-Hexachlorocyclohexane (β-isomer)		36.9	36.9	36.78
1*a*,2*a*,3*e*,4*e*,5*e*,6*a*-Hexachlorocyclohexane (γ-isomer)		35.9, 36.1, 36.5, 36.9.	35.9, 36.1, 36.5, 36.9.	35.78, 35.94, 36.37, 36.48, 36.83.
1*a*,2*a*,3*e*,4*e*,5*e*,6*e*-Hexachlorocyclohexane (α-isomer)		35.4, 35.5, 36.1, 36.4, 36.7.		35.60, 35.69, 36.23, 36.23, 36.47, 36.82.
1*a*,2*e*,3*e*,4*e*,5*e*,6*e*-Hexachlorocyclohexane (δ-isomer)		35.9, 36.1, 36.2, 36.7, 37.1.		35.95, 36.23, 36.26, 36.36, 36.82, 37.27.

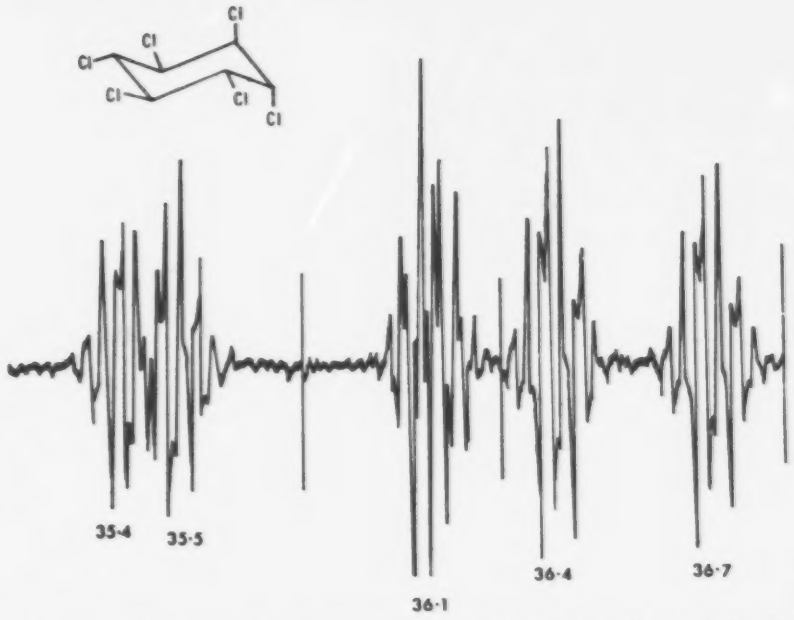


Figure 2. ^{35}Cl pure quadrupole resonances of 1,2,3,4,5,6-hexachlorocyclohexane (α-isomer) at 295 °K (resonance frequencies in MHz)

at 36.9 MHz in the spectrum of the β-isomer is consistent with the conformation of the molecule, that is, all chlorine atoms are equatorial and are in identical chemical environments around the cyclohexane ring. Four signals are observed in the NQR spectrum of the γ-isomer in the approximate ratio of 1 : 2 : 2 : 1 occurring at 35.9, 36.1, 36.5, and 36.9 MHz. The resonance signal at 36.9 MHz has been assigned to the single equatorial chlorine atom adjacent to two other equatorial chlorine atoms since it occurs at the same frequency as the signal in the β-isomer. The signal at lowest frequency, 35.9 MHz, has been assigned to the axial chlorine atom adjacent to the two remaining axial chlorine atoms. The signals at 36.1 and 36.5 MHz then have been attributed to the remaining chlorine atoms. From a consideration of molecular models, the resonance signal of the two axial chlorine atoms was assumed to be at higher frequency, 36.5 MHz since they appear to be more sterically crowded. On this basis, the two equatorial chlorine atoms each adjacent to one axial and one equatorial chlorine atom could be assigned to the resonance signal at 36.1 MHz.

We have extended Brame's interpretation of the NQR spectra of the β- and γ-isomers to the α- and δ-isomers of 1,2,3,4,5,6-hexachlorocyclohexane. Figure 2 illustrates the NQR spectrum of α-HCH. Five resonance signals have been observed in an approximate intensity ratio of 1 : 1 : 2 : 1 : 1. This isomer has two equatorial chlorine atoms adjacent to two other equatorial chlorine atoms. These chlorine atoms can be assigned to the high frequency signals at 36.4 and 36.7 MHz. The resonance signals would be closest in frequency to those observed for analogous equatorial chlorine atoms in the β- and γ-isomers. The small difference in the position of resonance for these two chlorine atoms is not believed to be significant. A difference in the resonance frequency in seemingly equivalent chlorine atoms is not uncommon and is observed rather frequently. This observation has not been interpreted with certainty, but has been attributed to crystal splitting or resonance shifts of chemically equivalent but physically inequivalent chlorine atoms due to different orientations of the molecules in the crystal lattice. Estimates of the influence of crystal effects on resonance shifts have been reported (*10*). The two axial chlorine atoms in the molecule may be assigned to the low frequency signals of 35.4 and 35.5 MHz. These assignments would be analogous to those made for the axial chlorine atoms in the γ-isomer (NQR resonance of 35.9 MHz). The remaining signal of 36.1 MHz which is more intense than the others may be assigned to the two remaining equatorial chlorine atoms. This assignment again, would be analogous to that made for the γ-isomer where two equatorial chlorine atoms each adjacent to an axial and an equatorial chlorine atom were assigned a resonance frequency of 36.1 MHz.

The NQR spectrum of the δ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane is somewhat more complex (Figure 3). The molecule possesses two equatorial chlorine atoms each having an adjacent axial and equatorial chlorine atom, three equatorial chlorine atoms each of which is adjacent to two other equatorial chlorine atoms and also a single axial chlorine atom adjacent to two other equatorial chlorine atoms. The signal of the latter chlorine atom is assigned to the resonance frequency of 35.9 MHz analogous to the previously discussed spectra. The signal centered at 36.1 MHz appears to be more intense than the remaining signals and could be due to the two equatorial chlorine atoms which are both adjacent to axial and equatorial chlorine atoms. It is interesting to note that if this assignment is correct, these chlorine atoms resonate at the same frequency as equivalent chlorine atoms found in the α- and β-isomers.

The three signals at highest frequency, 36.2, 36.7, and 37.1 MHz can now be assigned to the three remaining equatorial chlorine atoms. Here again, the reason for three separate signals for these chlorine atoms is not entirely clear but may

(10) P. J. Bray and R. G. Barnes, *J. Chem. Phys.*, **27**, No. 2, 551 (1957).

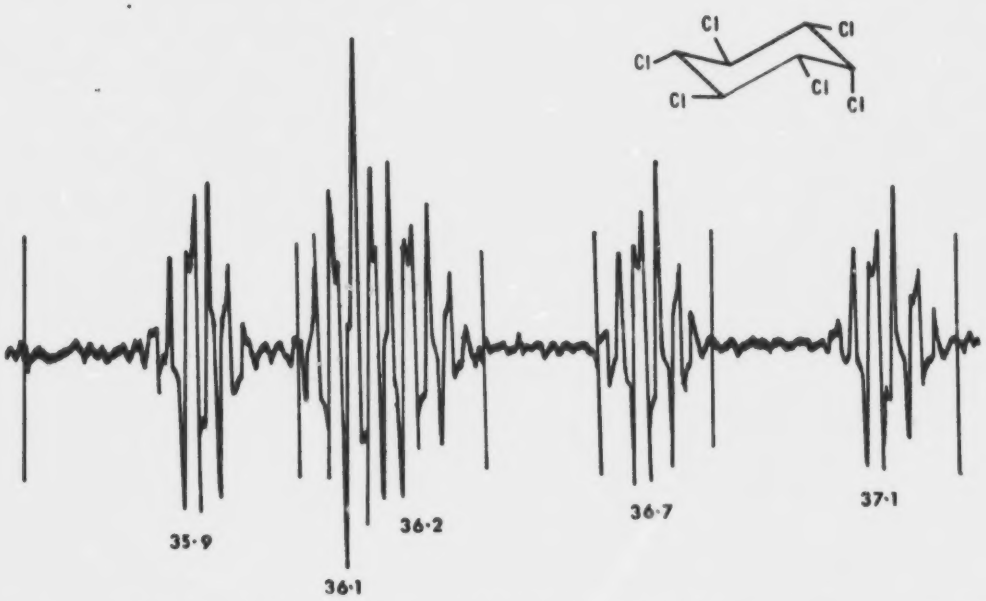


Figure 3. ^{35}Cl pure quadrupole resonances of 1,2,3,4,5,6-hexachlorocyclohexane (δ-isomer) at 295 °K (resonance frequencies in MHz)

be attributed to crystal splitting as discussed earlier for the γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane.

We have also obtained the ^{35}Cl pure quadrupole resonances of a number of cyclodiene chlorinated insecticides including heptachlor, heptachlor epoxide, endrin, aldrin, dieldrin, and α-chlordane. Figure 4 illustrates the structure and NQR resonances of heptachlor, representative of this group of pesticides. From the spectra-structure correlation chart given in Figure 1, it is possible to make some of the assignments. The resonance signals of 38.3 and 37.6 MHz may be assigned to the two vinylic chlorine atoms since they are known to occur at higher frequency than aliphatic chlorine atoms. The resonance frequencies at 37.0 and 36.5 MHz may then be assigned to the two chlorine atoms of the dichloromethylene group. As discussed earlier, spectra-structure correlations would seem to indicate that resonances of dichloro-substituted carbon atoms occur at higher frequencies than monochloro-substituted carbon atoms. On this basis, the signals of the chlorine atoms on the bridgehead carbon atoms are probably those centered at 36.2 and 36.4 MHz. The signal of the remaining chlorine atom on the cyclopentane ring is then centered at 34.1 MHz. Because of overlap of frequency ranges encountered in NQR studies for various structural types, it is possible that the assignment of the signals at 36.4 and 36.5 MHz could, in fact, be reversed.

The NQR spectrum of heptachlor epoxide is very similar to that of heptachlor and exhibits the expected seven signals. The introduction of an epoxide ring in the molecule results in some minor shifts in the resonance frequencies with the greatest change occurring in the resonance frequency of the chlorine atom on the cyclopentane ring. Following the reasoning outlined above for heptachlor, the assignments of the resonance frequencies of the chlorine atoms can be made as follows: vinylic chlorine atoms, 38.4 and 37.3 MHz; dichloromethylene bridge chlorine atoms, 36.8 and 36.6 MHz; bridgehead chlorine atoms, 36.2 and 36.1 MHz; and finally

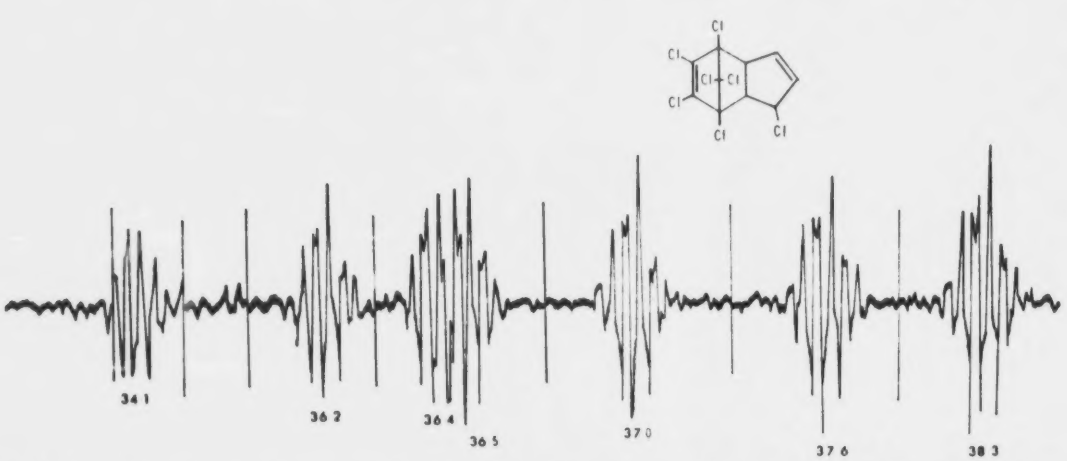
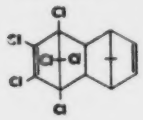
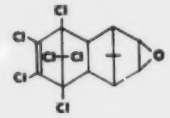
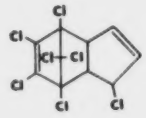
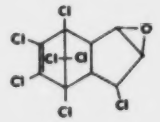
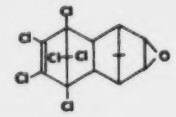
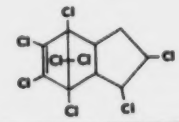


Figure 4. ^{35}Cl pure quadrupole resonances of 1,4,5,6,7,8,8-heptachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene (heptachlor) at 295 °K (resonance frequencies in MHz)

Table II. ^{35}Cl Pure Quadrupole Resonances of Some Cyclodiene Chlorinated Pesticides (in MHz at Ambient Temperature)

Compound	Structure	Resonance frequency
1,2,3,4,10,10-Hexachloro-1,4,4*a*,5,8,8*a*-hexahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene (Aldrin)		37.9
1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene (Dieldrin)		35.9, 36.1, 36.6, 37.5, 37.8, 38.1.
1,4,5,6,7,8,8-Heptachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene (Heptachlor)		34.1, 36.2, 36.4, 36.5, 37.0, 37.6, 38.3.
1,4,5,6,7,8,8-Heptachloro-2,3-epoxy-2,3,3*a*,4,7,7*a*-hexahydro-4,7-methanoindene (Heptachlor epoxide)		34.9, 36.1, 36.2, 36.6, 36.8, 37.3, 38.4.
1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo*,*endo*-5,8-dimethanonaphthalene (Endrin)		35.9, 36.1, 36.3, 37.0, 37.7.
cis-1,2,4,5,6,7,8,8-Octachloro-2,3,3*a*,4,7,7*a*-hexahydro-4,7-methanoindene (α-Chlordane)		33.7, 35.5, 36.3, 36.4, 36.6, 37.1, 37.8, 38.5.

the chlorine atom located on the five-membered ring may be assigned the resonance signal at 34.9 MHz. Six resonance signals corresponding to the six chlorine atoms present in the molecule are observed in the NQR spectrum of dieldrin. In this case, the same reasoning may be applied in assigning the various signals as was used for heptachlor and heptachlor epoxide. The high frequency signals of 37.8 and 38.1 MHz may be assigned to the two vinylic chlorine atoms. The two signals of 36.6 and 37.5 MHz may be assigned to the chlorine atoms of the dichloromethylene bridge and finally the signals of the bridgehead chlorine atoms are found at 35.9 and 36.1 MHz.

The NQR spectrum of endrin exhibits five major resonance frequencies as indicated in Table II. The assignments can be made as follows: both vinylic chlorine atom resonances appear at 37.7 MHz (in the NQR spectrum, this signal appears more intense than the others and may reasonably be assigned to two chlorine atoms), the bridge dichloromethylene chlorine atom resonances occur at 36.3 and 37.0 MHz, and the remaining bridgehead chlorine atom resonances are found at 35.9 and 36.1 MHz. Finally, the NQR spectrum of α-chlordane shows the expected eight resonances corresponding to the eight chlorine atoms found in the molecule. The assignments can be made in a fashion similar to that reported for the other members of this series with the vinylic chlorine atoms exhibiting high frequency resonances at 38.5 and 37.8 MHz. The dichloromethylene resonances are located at 36.6 and 37.1 MHz; the bridgehead chlorine atom resonances are found at 36.3 and 36.4 MHz; and, lastly, the cyclopentyl chlorine atoms show resonance signals of 33.7 and 35.5 MHz.

The preceding discussion illustrates the applicability of the NQR technique in structure determinations of organic molecules. The compounds reported, however, represent somewhat idealized cases, and it should be emphasized that even some of the assignments reported here are tentative. Unambiguous assignments must await appropriate theoretical studies or in the case of the more complex structures, the study of NQR spectra of suitable derivatives. Frequently, difficulties arise in obtaining adequate spectra for study. The NQR spectrum of aldrin, for example, exhibits one resonance signal at 37.9 MHz. From other observations in the cyclodiene series of insecticides reported here, it would be unlikely that all of the chlorine atoms resonate at the same frequency. A more likely explanation is that, because of instrumental insensitivity, the signal of only one or possibly two chlorine atoms is being seen. Another difficulty of the technique which has been encountered is the complete absence of signals from some polychlorinated compounds. These and other limitations may, however, be minimized with the development of more sensitive instrumentation and as improvements in sample handling techniques are made.

ACKNOWLEDGMENT

The authors are indebted to the Wilks Scientific Corp. for an advance copy of the First Edition of "NQR Bibliography," and to W. L. Truett for helpful discussions.

RECEIVED for review October 31, 1968. Accepted December 13, 1968. Presented in part at the 154th National Meeting, American Chemical Society, Chicago, Ill., September 1967, Division of Agricultural and Food Chemistry. Commercial sources and trade names are provided for identification only. Their mention does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

PB 275 982

PUBLICATION

216

PESTICIDES PROGRAM
National Communicable
Disease Center
Atlanta, Ga. 30333

FROM:

LECTURES ON GAS CHROMATOGRAPHY, 1966

edited by L.R. Mattick and H.A. Szymanski
Plenum Press, New York
pp. 121-136, 1967

Analytical Methods for Pesticides

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INTRODUCTION

The analysis of pesticide residues presents unusual problems because of the small amounts of materials that must be measured and the large amounts of interfering substances that must be removed. Tolerances for all compounds used on edible crops are set by the Food and Drug Administration. For compounds that possess low toxicity, tolerances are relatively high. Thus, residues of 7 ppm of DDT are permissible. However, crops containing a few hundredths of a ppm of endrin can be condemned. It is often necessary to analyze for much smaller residues than these in environmental health studies, since residues of pesticides in water at the parts per trillion (ppt) level are significant because aquatic flora and fauna are able to accumulate them against concentration gradients. In general, it is necessary to be able to analyze for pesticides at the (.01 to 10 ppm level in foods and animal tissues and at the ppt or ppb levels and upward in environmental pollution studies.

SAMPLE PREPARATION

Within recent years very sensitive methods of detection have been developed. Even so, it is necessary to concentrate

samples to a high degree prior to analysis. At the same time, the pesticides must be isolated from various interfering materials that occur in the environment. The procedures by which these objectives are achieved are called extraction and cleanup. These operations are basic to residue analysis, and must be accomplished quantitatively for the results to be meaningful. The methods described below are particularly applicable to chlorinated hydrocarbons and allied compounds. Modified procedures must often be employed for the analysis of organic phosphates, pesticide metabolites, and herbicides of the 2, 4-D class. (See "Guide to the Analysis of Pesticide Residues" [1] for details of methods.)

Extraction

The first step in preparing a sample for analysis is to extract the pesticide from it with an organic solvent. The solvent used may be polar, nonpolar, or a mixture of polar and nonpolar solvents. The choice will depend upon the nature of the sample and the polarities of the pesticides to be extracted. After extraction, the organic solvent containing the pesticides is evaporated to recover the residue. The residues obtained from water (1 liter) and tissue (100 g) samples may weigh as little as 1 mg to 1 g. This reduction in mass is made possible by the fact that many samples (excluding soils, fats, etc.) contain 90% or more water, most of which does not partition into the organic solvent. Also, many cellular constituents including cellulose, proteins, amino acids, carbohydrates, and related compounds are sparingly soluble in most organic solvents. However, most pesticides dissolve in polar and/or nonpolar organic solvents and hence are separated from the bulk of the sample. On evaporation of the organic solvent the pesticides are concentrated. This reduces the amount of material that must be handled in subsequent steps of the analytical procedure.

Cleanup

Many compounds other than pesticides that can be present in water, soil, and tissues are extractable with organic solvents.

These include industrial pollutants, lipids, pigments, and other nonpolar to moderately polar compounds. It is sometimes possible to analyze for pesticides in water and soil extracts without further separation from other compounds. With most extracts, cleanup (purification) is required to reduce the weight of the residue and remove substances that interfere in the analytical step. A variety of cleanup procedures have been utilized, but only a few are universal enough in scope to be applied to a wide variety of situations. These are cleanup by partition, by chromatography, and by saponification. In the partition method, the residue obtained after extraction is shaken in a separatory funnel with a mixture of two immiscible solvents. A nonpolar solvent (usually hexane or petroleum ether) and a polar solvent (usually acetonitrile) are always employed. Fatty materials dissolve in the nonpolar solvent, while most pesticides are partitioned into the polar phase. The two layers are then separated, and the pesticide residue is recovered by evaporation of the polar solvent. This procedure is very useful for the separation of pesticides from large amounts of oils and fats. However, it is not efficient for the removal of polar lipids, plant pigments, and other interfering compounds. Consequently, an adsorption step is usually required to remove these materials. This is carried out by dissolving the residue in an organic solvent and adding adsorbent or by percolating the solution through a column of adsorbent packed in a chromatographic column. Generally, adsorbents are chosen that possess high affinities for plant pigments and related compounds, but do not adsorb pesticides.

Samples that contain large amounts of fats and oils can be cleaned up efficiently by saponification. The samples are digested with alcoholic potassium hydroxide to convert triglycerides to soaps and glycerine. The solution is quenched with water and the pesticides extracted with an organic solvent such as petroleum ether. The soaps remain in the aqueous phase, which is discarded. This method is not as generally applicable as partition and column chromatography since many pesticides are destroyed by alkali. However, samples containing dieldrin, endrin, and aldrin can be cleaned up by this method. DDT is dehydrochlorinated to DDE and can be measured as such.

DETERMINATIVE METHODS

After the pesticide has been extracted from the sample and interfering compounds removed, the residue is ready for analysis. Formerly, specific methods were used for each individual pesticide in order to establish its identity and determine the amount present. These are usually colorimetric tests that are based on causing the pesticide to react with a reagent to produce a chromophore. The intensity of the color is then measured and the amount of pesticide present is computed from the color obtained on solutions of standards of known concentration.

During the past ten years colorimetric methods have been gradually superseded by chromatographic procedures in which a number of pesticides can be analyzed in the same operation. Chromatography per se is a technique for separating complex mixtures of compounds rather than analyzing for them quantitatively. However, when chromatography is used in conjunction with quantitative measuring systems, the combined operation provides more information to the residue analyst than any other technique available. Three forms of chromatography are currently in use: paper, thin-layer, and gas. The use of paper chromatography is declining while that of thin-layer chromatography is increasing. However, gas chromatography is superior to both of these methods with respect to resolution and sensitivity.

Paper Chromatography

To carry out paper chromatography, the sample is extracted and purified as described above and an aliquot of the residue is spotted near one end of a strip of filter paper. The end (lower) of the filter paper nearest the point where the sample was spotted is then dipped in a reservoir containing an organic solvent, the reservoir and filter paper being contained in an airtight jar or box. The solvent moves up the filter paper by capillarity, carrying the pesticide with it. In most cases, the

pesticides move up the paper at slower rates than the solvent because they are partitioned between the stationary phase (paper) and the mobile phase (solvent). Also, the individual pesticides are usually carried along by the solvent at different rates because their partition coefficients between the stationary phase and mobile phase vary from compound to compound. The paper is removed from the jar or chromatographic cabinet shortly before the solvent front reaches the top of the paper. By this time, the pesticides are spread out along the longitudinal axis of the paper as discrete spots. The distance between the point of sample application and the center of each spot, divided by the distance between the point of application and the solvent front at the termination of the run, gives the R_f value of the compound. Each pesticide possesses a characteristic R_f value under any given set of chromatographic conditions. However, some of these may approach each other closely. If the R_f values are widely separated, resolution will be good; if not, it will be poor. The resolution achieved for any pair of pesticides can be adjusted by changing the solvent system or pretreating the paper. An example of the separation of pesticides by paper chromatography is shown in Fig. 1.

It is not possible to detect the presence of compounds on the paper unless it is chemically treated. Usually, the paper is sprayed with a reagent that is specific for groups of compounds. Thus, a spray containing silver ion will serve to detect chlorinated hydrocarbons. The silver ion reacts with these compounds to yield silver chloride, which darkens on exposure to light. Similarly, specific reagents can be used for the detection of organic phosphate insecticides. Thus, it is possible to separate a series of related compounds on paper and to detect them all by spraying the paper with a reagent. This makes it possible to analyze simultaneously for a number of compounds even when the history of the sample is unknown. Paper chromatography is extremely useful as a screening test and has some advantages over gas chromatography for this purpose. The equipment is inexpensive, and a large number of samples can be run simultaneously in a single cabinet.

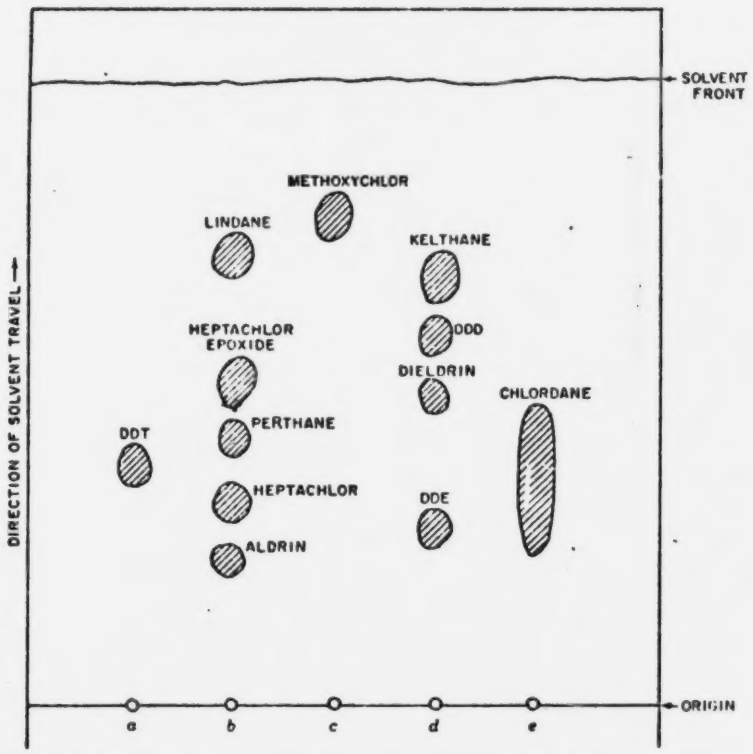


Fig. 1. Paper chromatogram showing: (a) migration of DDT, (b) separation of mixture of five pesticides, (c) migration of methoxychlor, (d) separation of mixture of four pesticides, and (e) streaking of chlordane.

Thin-Layer Chromatography

Thin-layer chromatography closely resembles paper chromatography except that the stationary phase used to retard the migration of the pesticides is an adsorbent such as silica gel or alumina rather than paper. The adsorbent (or active solid) is mixed with a binder and slurried in a solvent. The slurry is then deposited in a thin layer on a glass plate, and the

surface is smoothed with a doctor blade. The plate is dried to remove the solvent. At the end of this process, the adsorbent is bound firmly in a thin layer to the surface of the glass plate. A sample containing pesticides is applied to the lower end of the plate and the solvent is permitted to evaporate. The bottom end of the plate is then immersed in a reservoir of solvent contained in a sealed cabinet. As in paper chromatography, the solvent migrates by capillarity along the thin layer of active solid, carrying the pesticides with it at different rates. When the solvent front approaches the top of the plate, the chamber is opened and the plate is removed and dried. The location of the spots on the plate is determined by spraying with specific reagents as described for paper chromatography. The advantages of thin-layer chromatography over paper chromatography are that it is rapid, the properties of the stationary phase can be varied over a wide range by the choice and pretreatment of the active solid, and it is more sensitive. Thin-layer chromatography is also a very useful supplementary procedure to clean up samples prior to gas chromatography.

Gas Chromatography

Gas chromatography is the most useful single technique presently available to the residue analyst. It differs from paper and thin-layer chromatography in that a permanent gas such as nitrogen, argon, or helium is used as the mobile phase. The stationary phase is prepared by impregnating an inert solid with a nonvolatile organic liquid. A free-flowing powder is formed that is then packed into a glass tube usually $\frac{1}{4}$ in. in diameter and 6 ft long. The tube is installed in a heated cabinet and a stream of gas is forced through it at a constant rate. A solution containing a mixture of pesticides is injected into the hot column at the end nearest the gas inlet. The pesticides vaporize and are carried through the column by the moving gas. They travel through the column at different rates, owing to differences in their partition coefficients between the

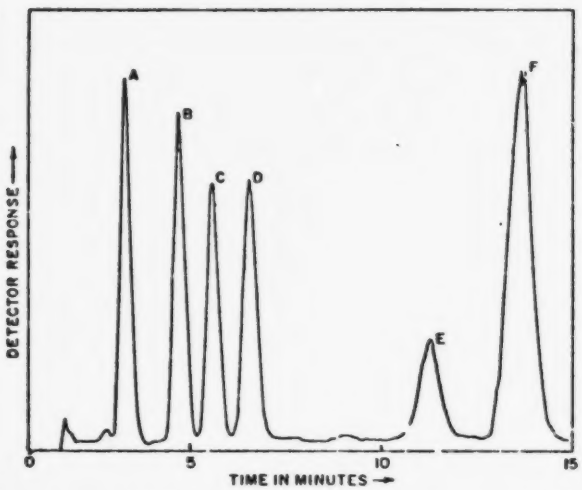


Fig. 2. Typical gas chromatogram obtained on a mixture of pesticide standards.

liquid stationary phase and the mobile gas phase. Consequently, they emerge from the far end of the column at different times and pass through a detector that automatically measures their concentrations. The response of the detector is fed into a strip-chart recorder so that the presence of each compound is registered as a peak and is measured as it emerges from the chromatograph. A typical gas chromatogram obtained on standard pesticides is shown in Fig. 2. The distance of each peak from the origin is known as the retention time and is characteristic for each pesticide. The area under the peak is proportional to the amount of compound passed through the detector. Thus, through the use of gas chromatography, it is possible to obtain both a qualitative and quantitative measure of the pesticides present in the sample.

The gas chromatographic method provides very high resolution combined with excellent sensitivity and specificity. Thus, complex mixtures containing lindane, heptachlor, aldrin, heptachlor epoxide, and DDT can be resolved in a single operation. As little as 10 picograms (pg) to 10 nanograms (ng)

of each compound can be detected, sensitivity being dependent on the type of detector used, the compound, and the level of background noise.

Several detectors are available that are capable of indicating the presence of pesticides with very high sensitivity and selectivity. The most sensitive of these is the electron capture detector. It is capable of measuring some pesticides in amounts as low as 10 pg. This detector yields extremely high responses to halogen-containing compounds and some organic phosphates but is relatively insensitive to many compounds that do not contain halogen. This reduces background noise and makes it possible to detect peaks arising from pesticides, even when impurities are present in the sample. However, specificity is not absolute. Many oxygenated hydrocarbons, particularly those containing carbonyl groups, give high responses to electron capture. Thus, it is not always possible to discriminate between peaks that arise from small amounts of chlorinated hydrocarbons and those that arise from larger amounts of oxygenated compounds.

When the extreme sensitivity of the electron capture detector is not required, the use of the microcoulometric detector is preferred because of its high degree of selectivity and the negligible background produced by most impurities. The microcoulometer is capable of detecting halogen and sulfur with absolute specificity. Thus, when a peak is observed on the gas chromatogram, it is certain that the compound giving rise to it contains the element being monitored. This minimizes the chances of confusing peaks arising from impurities with those of pesticides. The microcoulometer operates on the following principles. As each compound emerges from the chromatographic column, it passes through a combustion tube where it is burned with oxygen to yield water and carbon dioxide. If the compound contains halogen and sulfur, hydrogen chloride and sulfur dioxide are formed also. The gas stream then flows through a titration cell containing silver ion, which is maintained at a constant concentration electrochemically. Hydrogen chloride, if present, precipitates the silver ion stoichiometrically, and the current required to regenerate it from the

silver electrode is recorded as a gas chromatographic peak. Sulfur dioxide can be measured by replacing the chloride-sensitive titration cell with an oxidation-reduction cell. Using the model C-200 microcoulometer, as little as 10 ng of chlorine or sulfur can be measured. Hence, the procedure is more sensitive than any other currently available, with the exception of the electron capture detector.

SPECIAL PROBLEMS IN RESIDUE ANALYSIS

The methods described above are generally applicable to chlorinated hydrocarbon insecticides and allied compounds. However, a fairly large number of chemicals are now used in agriculture that do not fall into these categories. Some of these present special problems in analysis that require modification of the standard techniques used for chlorinated hydrocarbons.

Organic Phosphates

Generally applicable methods for the analysis of organic phosphates currently available are not very satisfactory. Extraction of the parent compounds from most media is no more difficult than in the case of chlorinated hydrocarbons. However, oxidation and hydrolysis products are formed rapidly on exposure of these compounds to the environment. These are more polar and water soluble than the parent compounds and, consequently, are more difficult to extract. Difficulty has been encountered in extracting many of these materials from soil, either due to decomposition or irreversible binding to soil particles. General procedures for cleaning up these extracts are also presently unavailable, although they are being worked on in many laboratories. Some phosphates decompose on or are bound irreversibly by the adsorbents used for column chromatography of chlorinated hydrocarbons. Several column methods have been developed specifically for them, but they have been applied to only a limited number of compounds.

After extraction and cleanup, paper chromatography is commonly used for separation and analysis. Adequate resolu-

tion is obtained, and it is possible to detect a number of compounds specifically through employment of the appropriate chemical reagents. Thin-layer chromatography is just coming into use for the analysis of organic phosphates, and the preliminary results available look very promising.

Gas chromatography also can be used for the measurement of phosphates. Column conditions have not yet been established that will permit the elution of all of the phosphates quantitatively. However, recoveries are reasonably good in many cases. More difficulty is experienced in chromatographing some of the oxidation and hydrolysis products of phosphates. However, the chances are good that these can be converted to stable compounds by reduction and methylation.

Several detection systems are currently being evaluated for the selective detection of phosphates. In one of these, the organic phosphates are reduced to phosphine, and this gas is measured selectively with the microcoulometric titration system [2]. Another procedure makes use of a flame ionization detector in which a coat of a sodium salt is fused onto the electrode. This enhances the responses obtained to organic phosphates compared to those yielded by hydrocarbons and chlorinated hydrocarbons [3]. Finally, a method has been developed by McCormack et al. [4] in which the column effluent is analyzed by emission spectroscopy with the monochrometer set at the phosphorus line at 2539 Å.

Herbicides

Herbicides such as 2,4-D and 2,4,5-T are difficult to extract from most samples. Because they are anionic, the substrate usually must be acidified prior to extraction and a polar solvent used. These compounds can occur in a number of forms in the sample, so provision must be made for all of these in order to obtain quantitative results. Thus, esters of 2,4-D as well as salts are used in agriculture. These esters can hydrolyze to the free acid, particularly when taken up by plant tissues. Moreover, the free acids can become conjugated with sugars or other metabolites. These conjugates are impossible to extract by conventional procedures; there-

fore, they should be hydrolyzed prior to extraction. Many published methods do not provide for this. For extraction to be quantitative, it is necessary to provide for recovery of the free acid, esters, and possible conjugates with naturally occurring metabolites. Also, it has been found that proteins present in some samples can result in poor recoveries of 2,4-D. Evidently, they are capable of binding 2,4-D at cationic sites, thus shifting the partition coefficient in the direction of the aqueous phase. This effect can be eliminated by hydrolyzing the protein enzymatically prior to extraction.

Gas-liquid chromatography employing the microcoulometric or electron capture detector is the most satisfactory method currently available for the analysis of the compounds. The acids are usually converted to methyl esters by treatment of the residue with diazomethane or methanol-BF_3. Yields of the esters are satisfactory, and they chromatograph without decomposition.

IDENTIFICATION BY INFRARED ABSORPTION

Excellent circumstantial evidence leading to the identification of unknown pesticides can be obtained by gas chromatography or a combination of gas and thin-layer chromatography. However, further confirmation of identity by optical methods is generally required for absolute proof. Identification by infrared spectrophotometry is the most satisfactory technique currently available. Other methods are sometimes employed, but they have not received general acceptance. These include mass spectrometry, nuclear magnetic resonance spectrometry, ultraviolet spectrophotometry, and sometimes combinations of these. Some of these techniques lack the specificity required in pesticide residue analysis. Others require large samples and highly skilled personnel for operation and interpretation of results, are high in cost, or are not developed to the point where procedures can be recommended for routine laboratory use. Infrared spectrophotometry is therefore the most satisfactory method presently available. However, the limited

quantity of purified pesticides recoverable from typical residue samples often presents considerable problems.

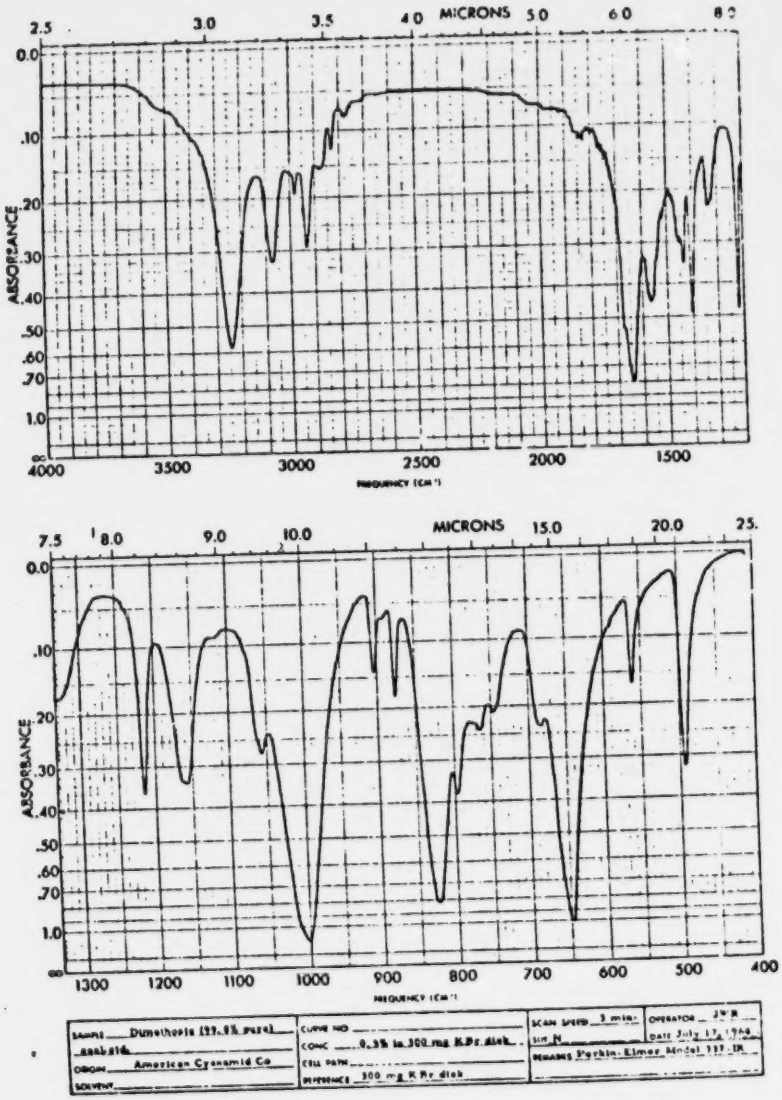
Therefore, the infrared spectrophotometer serves primarily as a supplement to the more sensitive quantitative instruments such as the gas chromatograph. It provides evidence by means of recorded spectra which, like fingerprints, are used for unequivocal identification of compounds. The spectrum of an unknown is compared with those of standards; identity is established if one of the traces is superimposable with that of the unknown. Even when complete matching of all details of the traces is not achieved, it is possible to identify or eliminate candidate functional groups, since most groups possess characteristic absorption bands.

The application of infrared spectrophotometry to residue analysis is dependent on obtaining a highly purified sample by chromatography. Because the most severe limitation up to now has been lack of sensitivity of the infrared spectrophotometer, much less has been published on residue analysis than on analysis of formulations where much larger quantities are available. However, improvements in instrumentation and refinements in sample isolation techniques are obviously brightening the outlook for fuller exploitation of infrared techniques in residue work.

Samples of unknown pesticides are isolated by gas or thin-layer chromatography and prepared for infrared analysis by incorporating them in KBr microdiscs or placing them in microcavity cells, with or without an organic solvent. The spectra are then obtained in the usual way and compared with the spectra of known pesticides in order to obtain the best matching pair.

For best matching, spectra of both unknowns and standards should be obtained on the same type of instrument, preferably on the same instrument. In this way, the spectra can be compared directly by superimposing the traces. The spectrum of the insecticide dimethoate is shown in Fig. 3.

Many factors contribute to the type of spectrum obtained, sample concentration being one of these. If the sample is too dilute, only the largest peaks will be seen, while if the sample


2.5
3.0
3.5
4.0
MICRONS
5.0
6.0
0.0
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.20
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.70
1.0
ABSORBANCE
4000
3500
3000
2500
2000
1500
7.5
8.0
9.0
10.0
MICRONS
15.0
20.0
25.
1300
1200
1100
1000
900
800
700
600
500
400
SAMPLE Dimethoate (99.8% pure)
CURVE NO
SCAN SPEED 3 min.
OPERATOR
CONC 0.3% in 300 mg KBr disk
ORIGIN American Cyanamid Co
CELL PATH
REMARKS Perkin-Elmer Model 337 IR
SOLVENT
REFERENCE 300 mg KBr disk

Fig. 3

is too concentrated, larger peaks will be off-scale. In either case, it would be hard to compare the spectra with those of known compounds, and important spectral information would be lost. Thus, it is often necessary to run a trial spectrum and then adjust the sample size, solution concentration, or film thickness according to the results obtained.

Another important factor when comparing spectra is to present the sample and standard in the same way. Thus, an unknown sample prepared in a KBr disc should be compared to known standards with spectra obtained in KBr discs and films compared with films. Solutions should always be prepared in the same solvents, and spectra of these solvents should also be obtained in cells of the same size so that the analyst is aware of any possible peak interferences. In brief, the unknown and the standard compounds should be handled by techniques as similar as possible to obtain the best correlation of spectra. Complete records of the method of preparation and presentation of the sample should be included on the spectra of standard compounds and unknown samples.

Despite all possible care in the preparation of unknowns and standards, the spectra may not superimpose exactly. This can also occur if uniform sample preparation is not possible. However, comparisons of certain absorption peaks may lead to positive identification. The most important peaks are the largest peaks arising from absorption frequencies of functional groups, so that these should be examined with particular care. When the spectra are presented in different forms it is best to make a list of the frequencies of the major peaks. One system is to record in decreasing order of peak size the frequencies of the largest peaks of the unknown sample. The frequencies of the comparable peaks on the standard spectrum are then placed directly opposite. Correspondence of the major peaks with respect to size, order, and frequency constitute positive identification. It is generally agreed that at least the three major peaks must agree before proof of identity is acceptable. These three peaks should not arise from the same functional group, such as C=O, P—O, or C—Cl. Also, these major peaks should not be those arising from C-H vibrations. C-H stretching frequencies occur around 3000 to 2700 cm^{-1}, while

C-H deformation frequencies occur around 1450 to 1350 cm^{-1}. Thus, major peaks in these regions should not be considered.

When spectra are not completely superimposable and the method of peak comparison is used, it is advisable for the analyst to have some knowledge of spectral interpretation in regions of functional group absorption. Absorption maxima in different regions of the infrared indicate different types of vibrations between different atoms in the molecule. These regions, and types of absorption bands produced, are specific enough from molecule to molecule so that much can be learned about unknown samples without references to standard compounds. Often the molecule, by the spectrum alone, can be positively identified by a spectroscopist well trained in this field. Thus, organic phosphates have a characteristic absorption band dependent upon vibration between the phosphorus atom and other atoms or groups. The P-O (free) vibration produces a strong absorption maximum in the region around 1350 to 1250 cm^{-1}, while P-O (hydrogen bonded) produces a strong maximum from 1250 to 1150 cm^{-1}. These are just two examples of many bands that might occur, depending upon the groups attached to phosphorus atoms and the molecular interactions produced. All of the major peaks of a spectrum must be studied and interpreted, and interrelations must be noted before an identification can be made. When the analyst has some knowledge of these absorption bands and the interactions that may cause them, he is in a much better position to interpret any doubtful spectra intelligently.

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PB 275 983

The Electron-Capture Gas Chromatography of Paradichlorobenzene Metabolites as a Measure of Exposure

PESTICIDE PUBLICATION
3 5 7
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Paradichlorobenzene has been used extensively as a household moth control agent and as a deodorant for many years, and it is considered to be safe and suitable for these purposes. However, cases of poisoning attributed to paradichlorobenzene have been reported (1-4). A convenient method for determining exposure to this halobenzene is important in evaluating any hazards involved in its use. The halobenzenes are metabolized mainly by oxidation to phenols, quinols, catechols and related compounds, or by forming mercapturic acids.

Various workers have reported (2,5) that the primary metabolites of paradichlorobenzene (PDB) are 2,5-dichlorophenol (DCP) and 2,5-dichloroquinol (DCQ). They are excreted in the urine as glucuronide and ethereal sulfate conjugates. PDB differs from its o- and m-isomers in that none of its metabolites are mercapturic acids (6). Furthermore, unlike PDB, the o- and m-isomers have metabolites that are qualitatively similar to those of the monochlorobenzenes although they differ completely quantitatively (7).

The high volatility and reactivity of PDB complicates its detection; however, the determination of its less volatile and reactive metabolites, DCP and DCQ, would not be as difficult. Existing methods (8-13) for the determination of halogenated phenols, quinols, etc., including some GLC (gas liquid chromatography) methods, are less sensitive and dependable than that desired. This paper describes a method which successfully employs gas chromatography with electron capture detection for the measurement of PDB and its major metabolites.

Experimental Procedure

Animal Dosing

New Zealand white rabbits were given a single dose of 0.5 g/kg of PDB [dosage identical to that used by Azouz et al. (5)] by stomach tube; blood samples were obtained 1 day, 2 days, and 3 days following the single dose and urine samples were collected in the same manner as that of Azouz et al. (5). Blood and urine samples were also obtained from control rabbits. All blood samples were immediately centrifuged and refrigerated prior to analysis.

Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 4, 1970, published by Springer-Verlag New York, Inc.

Methods

A 5-ml urine sample was acidified with 0.5-ml of concentrated hydrochloric acid (HCl). This solution was shaken at room temperature for 30 minutes. The acidic solution was then extracted with two 5-ml portions of pesticide quality benzene. In some samples it was necessary to add NaCl or $(NH_4)_2SO_4$ to prevent or reduce emulsion formation. The combined benzene extracts were dried over anhydrous sodium sulfate (Na_2SO_4). The necessary concentrations for GLC analysis were made by diluting portions of this dried solution.

A 5-ml whole blood sample was treated in the following manner. The sample was centrifuged at 10°C and 600 x g to separate the red blood cells from the plasma. The plasma layer (2.0 to 2.5 ml) was extracted with two 3-ml portions of pesticide quality benzene. The combined extracts were then dried over anhydrous Na_2SO_4 and subjected to GLC analysis without further dilution.

GLC Columns

The GLC analyses were made on a Varian Aerograph model 2100 gas chromatograph equipped with a tritium detector. Two columns were required for the complete analysis, and these were used interchangeably with the tritium detector and variations in column temperature for the desired analysis. PDB and DCP were analyzed directly using 15% FFAP (half ester of 2-nitroterephthalic acid-carbowax) as the stationary liquid phase with 40 to 50 mesh Chromasorb W as the supporting phase. The column material was prepared by dissolving 15 grams of FFAP in 250 to 300 ml of methylene chloride. The solution was transferred to a round bottom flask to which was added 85 grams of 40 to 50 mesh Chromasorb W. The slurry was freed of methylene chloride by rotary evaporation and the resulting solid material was dried overnight in an oven at 150°C. A 6' x 1/8" silanized glass column was then packed with this material.

DCQ was analyzed directly using 3% DEGS (diethylene glycol succinate) + 1% phosphoric acid on 30 to 60 mesh Chromasorb W. This column material was prepared by dissolving 3 grams of DEGS in 50 to 100 ml of acetone to which was added a solution of 1 gram of phosphoric acid in 50 ml of acetone. Ninety-six grams of 30 to 60 mesh Chromasorb W was added to the resulting solution. The slurry was freed of acetone by rotary evaporation, dried and packed in a 3' x 1/8" silanized glass column.

GLC Analysis

The gas chromatographic parameters necessary for each analysis are listed in Table I. Both columns were baked for 16 hrs. at 200°C prior to conditioning. Nitrogen was used as the carrier gas throughout the analysis.

Standard solutions (3 to 5 nanograms per microliter) of PDB, DCP and DCQ were prepared in suitable pesticide quality solvents (used throughout this work) and subjected to GLC analysis.

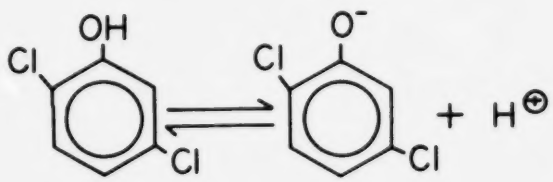
TABLE I

Gas Chromatographic Conditions

Compound	Column Material	Oven Temp. °C	Detector Temp. °C	Inlet Temp. °C	Flow Rate cc/min.
PDB	15% FFAP	140	200	225	45
DCP	15% FFAP	170	200	225	45
DCQ	3% DEGS + 1% H_3PO_4	170	200	225	45

Results and Discussion

Low volatility and high adsorption properties of phenolic pesticides has limited their direct analysis on silicone columns. It was observed in our laboratory that the phenolic and quinolic metabolites of PDB could be analyzed directly using polar liquid phases which permitted low adsorptivity (14,15). The ability of these materials to furnish protons or positive sites, thereby shifting the thermally influenced chemical equilibrium of the substance in the direction of its unionized (associated) form, permitted the direct analysis.



In the case of DCQ this was accomplished with good sensitivity while still retaining symmetrical peaks using a DEGS column with added phosphoric acid. In the case of DCP a higher degree of adsorptivity was achieved using FFAP. The availability of protons (positive sites) in FFAP is due to the electron withdrawing effect of the nitro group on the "free" carboxyl group. Table II presents the retention times and sensitivity of the method. The urine analyses gave 186 ppm of DCP and 2.6 ppm of DCQ with no detectable PDB. The numbers given are the average values obtained from two to four determinations. Typical chromatograms for standards, control urine and experimental urine are shown in Figures I-III. The overshoot shown on the chromatograms for the FFAP column diminished with repeated injections and did not significantly reduce the standing current of the gas chromatograph.

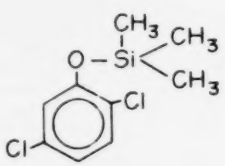
TABLE II

Gas chromatography data for PDB, DCP, and DCQ (using conditions shown in Table I).

Compound	Retention Time (min)	Nanograms Injected	% Deflection of Full Scale	Method Sensitivity (peak height)
PDB	1.2	5	65	0.38 ng/cm
DCP	7.3	5	45	0.55 ng/cm
DCQ	7.0	0.5	45	0.06 ng/cm

The presence of DCP in the urine was qualitatively confirmed by pretreatment of the sample to form the silyl ether derivative (I). The derivative was determined on a 3' aluminum column of 5% DC-200 on 60 to 80 Chromasorb G at 138° installed in a Varian Aerograph 1525 B gas chromatograph. Although quantification by this method was poor, this work provided information pertinent to the work-up procedure following silylation (16) of phenolic residues. For example, an aqueous extraction of the hexane silylation reaction solution affords a much cleaner chromatogram and does not significantly lower the concentration of the desired silyl ether through hydrolysis; however, hydrolysis did yield a siloxane (II) peak which could be used as an internal standard from which relative retention times could be calculated (I, RRT = 1.6 min; III, RRT = 6.8 min).

This silylation method was not successful in confirming the presence of DCQ in rabbit urine probably due to difficulty in silylating both hydroxyl groups quantitatively as well as the low concentrations of this metabolite present. However, standard solutions of DCQ were converted into a silyl derivative which was then chromatographed on the 5% DC-200 column. A relatively long retention time obtained for this derivative suggested that it might have been the monosilyl derivative (III).



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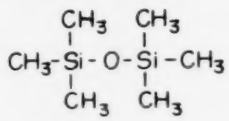

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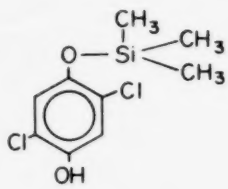
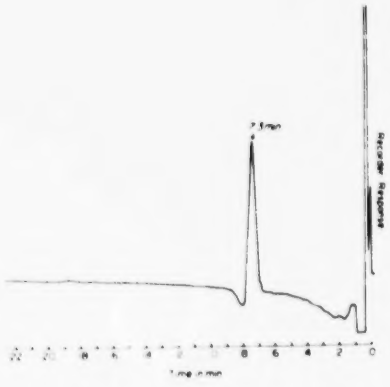
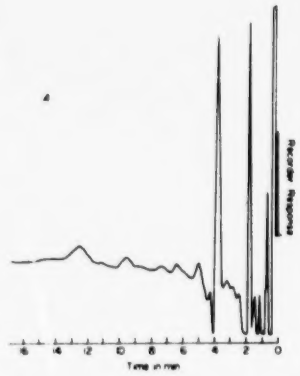
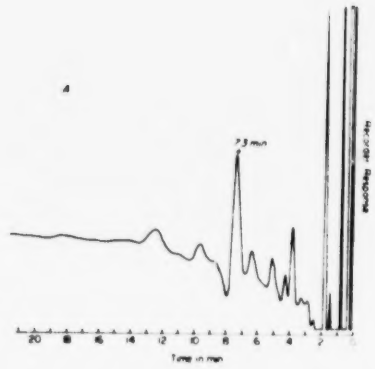
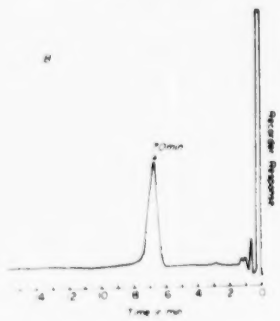


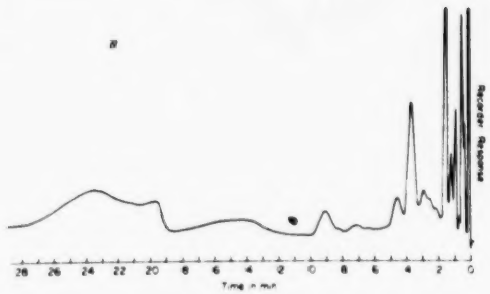
III











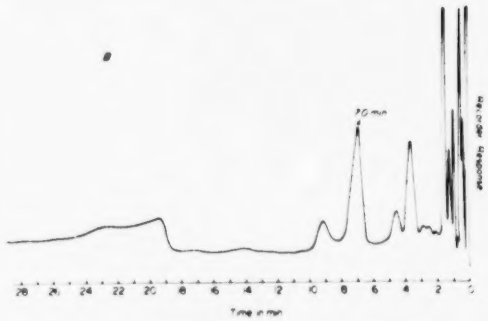


Figure I Standard Solutions of DCP and DCQ
A: 5 mg of DCP on 5% FFAP
B: 0.5 mg of DCQ on 3% DEGS/
% H_3PO_4

Figure II Control urine
A: 1 microliter on 5% FFAP
B: 5 microliters on 3% DEGS/
% H_3PO_4

Figure III Experimental urine
A: 1 microliter on 5% FFAP
B: 5 microliters on 3% DEGS/
% H_3PO_4

Previous workers (5) who were primarily interested in qualitative identification of PDB metabolites had used vigorous hydrolysis with refluxing concentrated hydrochloric acid (HCl) and steam distillation in order to free DCP and DCQ from its conjugates. These conditions would not only favor loss of the materials due to volatilization but would also facilitate further reaction and/or decomposition. Betts et al. (17) have shown that pentachlorophenol levels in rabbit urine are not significantly increased by hydrolysis with 5N-HCl before steam distillation beyond those levels obtained by steam distillation of the urine adjusted to pH 1; therefore, if conjugates form at all, they must be very labile to acid. In analogy with pentachlorophenol, the conjugates of DCP and DCQ might also be labile. Furthermore, Ishidate et al. (18) have shown that the electronically analogous N-glucuronide derived from aniline can be hydrolyzed on a column simply by eluting with 1N HCl. Spiked urine samples were subjected to a variety of extraction solvents and workup procedures, including those used by Azouz et al. (5), to obtain conditions affording the highest recovery percentages. However, none of the variations attempted gave a significant increase in the recovery percentages obtained by the simple and convenient procedure described in this paper.

TABLE III

% Recoveries from Spiked Samples

a) Urine

PDB	DCP	DCQ
78	92	85

b) Serum

94	87	0

As shown in Table III this extraction procedure failed to recover DCQ from spiked serum samples. Other extraction procedures as well were unsuccessful in recovering DCQ from serum suggesting that it may be tightly bound to blood components. This can be attributed to the apparent lability of DCQ to autooxidation as evidenced by increasing coloration of its standard solutions with time. The p-quinoidal system formed would greatly enhance the binding propensities. PDB nor its metabolites were found in any of the blood werum samples from dosed rabbits.

Although our control samples indicated that it was not necessary in our analysis to include a basic extraction followed by acidification and re-extraction as is normally done in the cleanup procedure for isolating phenolic metabolites, further cleanup may be desirable to increase the lifetime of the columns if these analyses are performed routinely. However, a significant loss of sensitivity did not occur with these columns after several months

of repeated injections under the conditions described in Table I.

No attempt was made to establish the percentage of the dose represented by the various levels of metabolites found, nor was any attempt made to determine the levels of glucuronide and ethereal sulfate conjugates before hydrolysis. Previous workers (5) have shown that PDB nor its metabolites are excreted in feces. In considering a practical application of the method, it would be difficult to determine the exact dosage an individual received from exposure; therefore, it would be difficult to ascertain the percentage of the dose excreted as metabolites. The method will hopefully permit determination of "tolerable" levels of exposure as measured by excreted metabolites prior to acquiring toxic symptoms. This more sensitive and convenient method for determining exposure to PDB via measurement of the major urinary metabolites may also be advantageous in elucidating the mode and mechanism of action of PDB, and other halobenzenes, and may have general application for the direct GLC determination of chlorinated phenols with electron capture detection.

Acknowledgment

The authors are grateful to Renate Kimbrough and Claudia Lewis for their helpful suggestions in preparing the manuscript. We also acknowledge the technical assistance of C. L. Thrasher.

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PB 275 984

A MULTICLASS, MULTIRESIDUE ANALYTICAL METHOD FOR DETERMINING PESTICIDE RESIDUES IN AIR

PESTICIDE PUBLICATION

716

682

Environmental Protection Agency

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A multiresidue method for chlorinated, organophosphate and *N*-methyl carbamate insecticides has been developed for use in the National Air Monitoring Program. The method involves partitioning and extracting the pesticides from the ethylene glycol trapping solvent with methylene chloride followed by fractionation and cleanup by elution through a silica gel column. The chlorinated compounds are determined by electron capture GC, phosphate compounds by flame photometric GC, and carbamates by electron capture GC after derivatization with pentafluoropropionic anhydride. Recovery data and limits of detectability are presented for 11 chlorinated, 7 phosphate, and 7 carbamate pesticides at high and low levels. It is expected that the method will be applicable to many other compounds not successfully determined by the present analytical procedure, and that it may be adaptable for the analysis of pesticide residues in foods and other environmental samples.

The National Air Monitoring Program (Enos *et al.* 1972; Yobs *et al.* 1972) for the determination of pesticide residues in air currently uses a collecting system composed of ethylene glycol in a Greenburg-Smith type impinger (Miles *et al.* 1970) interfaced with the Food and Drug Administration (FDA) multiresidue analytical procedure (FDA 1967). Limitations of this existing system (Thompson 1971) are that only those pesticides extracted from ethylene glycol with hexane and subsequently eluted from the Florisil column by ethyl ether-petroleum ether eluents are finally identified and quantitated. Numerous carbamate and phosphate pesticides are not determined by this system because of these limitations. The purpose of the present study was to devise a more comprehensive multiresidue method for chlorinated, phosphate and carbamate pesticides in ethylene glycol to overcome the diffifulties with the FDA multiresidue procedure in the air monitoring scheme.

Different approaches have been taken by other workers for multiresidue analysis of various samples. Abbott *et al.* (1970) determined 39 phosphate pesticide and metabolite residues in foods using three extraction procedures and cleanup by charcoal chromatography or solvent partitioning for seven commodity groups comprising the total diet.

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Presented at the 164th National ACS Meeting, Pesticides Division, New York, N.Y., August 27, 1972.

Archives of Environmental Contamination and Toxicology, Vol. 3, No. 1, 1975, © 1975 by Springer-Verlag New York Inc.

Final determination was by total phosphorus and/or CsBr thermionic-GC methods. Chlorinated pesticides in human tissue were determined by a micromethod employing a 1.6 g Florisil column eluted with 12 ml of hexane followed by 12 ml of 1% methanol in hexane (fraction 1) and then 12 ml more of 1% methanol in hexane (fraction 2) (Thompson 1971). Mills *et al.* (1972) employed three eluents for the improved cleanup and recovery of 50 chlorinated and phosphate chemicals in extracts of fats and oils. These consisted of 20% methylene chloride-hexane (v/v), methylene chloride – 0.35% acetonitrile – 49.65% hexane (v/v/v), and 50% methylene chloride – 1.5% acetonitrile – 48.5% hexane (v/v/v). Law and Goerlitz (1970) found microcolumns of alumina and silica to be satisfactory for the cleanup and recovery of 18 chlorinated and phosphate pesticides in water. The alumina was deactivated with 5% water and eluted with hexane followed by benzene-hexane (1:1), and the silica (5% water) was eluted in turn with hexane, benzene-hexane (15:85), benzene-hexane (1:1), and ethyl ether-hexane (2:8). Malathion, parathion and methyl parathion could not be successfully recovered from Florisil when a modified Mills procedure was attempted. Storherr *et al.* (1971) described a procedure in which a short charcoal column eluted with acetonitrile-benzene (1:1) was used for the cleanup of 41 phosphate pesticides and/or alteration products present in an acetonitrile extract of plant materials. Determination was by thermionic or FPD-GC. Holden and Marsden (1969) studied alumina and silica gel columns deactivated with 5% water and eluted with hexane for the cleanup and differential elution of 14 organochlorine residues present in animal tissues. Kadoum (1967, 1968, 1969) employed microcolumns of activated Fisher silica gel grades 923 and 950 to clean up clorinated and phosphate residues in plant, animal, soil and water samples. Johnson (1970) used microcolumns of silica gel grade 950 deactivated with 1% water and eluted with 10% and 60% benzene in hexane for the analysis of chlorinated pesticides in water. Leoni (1971) separated 50 chlorinated and phosphate pesticides and PCB's into four groups by chromatography on a silica gel microcolumn by elution in turn with *n*-hexane, 60% benzene in hexane, benzene, and 50% ethyl acetate in benzene.

Methylene chloride has been used by several researchers to extract residues of organophosphate pesticides, carbaryl, carbofuran and a series of seven *N*-methylcarbamate insecticides (Abbott *et al.* 1970; Porter *et al.* 1969; Butler and McDonough 1971).

Our preliminary experiments indicated that the use of methylene chloride for extraction and partitioning and silica gel containing 20% water for column chromatography would provide the best system, in terms of ability to recover the maximum number of pesticides and to provide cleanup and differential elution patterns, for the multiresidue analysis of the three classes of interest. This system also offers the greatest potential for adding other pesticides to the analytical scheme in addition to the limited number studied in the developmental work described below.

Experimental

Apparatus. A Micro-Tek MT-220 gas chromatograph was operated under the following conditions: (a) 6 ft × 1/4 in. U-shaped glass column packed with 5% GE-SE-30 on Chromosorb W (HP), 80-100 mesh; inlet, transfer line, and tritium foil EC detector

temperatures, 250°, 245°, and 210°C, respectively; for determination of organochlorine pesticides, the column temperature was 195°C and the nitrogen carrier gas flow rate was 70 ml/min; for determination of carbamates, the column temperature was 165°C and the flow rate was 30 ml/min. (b) 6 ft × 1/4 in. U-shaped glass column packed with 5% OV-210 on Supelcoport, 80-100 mesh; conditions as above except the column temperature was 180°C and the nitrogen carrier gas flow rate was 60 ml/min. (c) 6 ft × 1/4 in. U-shaped glass column packed with 5% OV-210 on Supelcoport, 80-100 mesh, Carbowax 20M treated (Ives and Giuffrida 1970); the nitrogen carrier gas flow rate was 60 ml/min; column, inlet, and transfer line temperatures were 180°, 235°, and 245°C, respectively; and the Melpar flame photometric detector (phosphorus mode) and detector base temperatures were 185° and 225°C, respectively.

Nitrogen evaporator with water bath maintained at 40°C, N-EVAP Model 10, Organomation Assoc., Worcester, Massachusetts.

Reagents. Nanograde® (Mallinckrodt) solvents: hexane, benzene, methylene chloride, acetonitrile, isooctane.

Anhydrous sodium sulfate, benzene-extracted.

Water, deionized and benzene-extracted.

Ethylene Glycol: 30 ml of methylene chloride is dissolved in 125 ml of redistilled ethylene glycol in a separatory funnel. The solution is extracted in turn with 30 ml of benzene and three 30-ml portions of hexane.

Keeper solution: 1% paraffin oil in hexane.

Pyridine, redistilled.

Silica gel: Woelm, activity grade 1, activated for 48 hr at 175°C. Deactivate by adding one ml of water to five g of silica gel in a tightly-capped vial. Mix on a Roto-Rack (Fisher) for two hours at setting 8. The performance of the deactivated adsorbent did not change over a period of at least five days if the unused portion was kept in a closed vial.

Pentafluoropropionic anhydride: Pierce Chemical Co., No. 6-5193. Keep refrigerated.

Phosphate buffer (pH7): three g of NaOH and 17 g of KH_2PO_4 in 200 ml of water, extracted with benzene.

Determination of pesticides in ethylene glycol. The procedure is based on a sample of 100 ml of ethylene glycol, which is the amount in one air-sampling impinger and is equivalent to 20 m^3 of air collected over a period of six hours. For analysis, the ethylene glycol is washed from the impinger, or from the bottle used to transport the sample to the laboratory, into a one-liter separatory funnel (Teflon stopcock) with a portion of 2%

Na_2SO_4 solution. The fiber-glass pre-filter from the air sampler can be added directly to the separatory funnel.

Extraction and silica gel chromatography. To 100 ml of ethylene glycol in the separatory funnel add 600 ml of 2% Na_2SO_4 and 40 ml of methylene chloride ($MeCl_2$). Shake vigorously for two min, venting pressure several times during the first 30 sec. Allow the phases to separate for 30-45 min with occasional swirling of the flask. Drain the $MeCl_2$ layer into a 45-ml centrifuge tube. Approximately 33 ml will be recovered from the 40 ml originally added when ethylene glycol purified as described is employed. Evaporate to a volume of about five ml under a stream of nitrogen. Add 10 ml of 2% Na_2SO_4, mix on a Vortex-Genie mixer at maximum speed for one min, and centrifuge to separate the layers. Remove and discard as much of the aqueous (top) layer as possible with a disposable pipette without removing any $MeCl_2$. Repeat the washing with two more ten-ml portions of Na_2SO_4 and after the third wash (discarding of most of the upper layer) add enough solid Na_2SO_4 to remove the last amounts of water. Transfer the $MeCl_2$ above the Na_2SO_4 to a 13-ml centrifuge tube with a disposable pipette. Wash the solid Na_2SO_4 in the 45-ml tube with three two-ml portions of $MeCl_2$ adding these to the 13-ml tube. The last time, plunge the disposable pipette into the solid salt to transfer as much of the remaining $MeCl_2$ as possible. Add five drops of keeper solution and evaporate the contents of the 13-ml tube just to dryness under nitrogen.

Prepare the silica gel column by lightly plugging a size 22 Chromaflex glass tube with glass wool, adding 1.0 g of deactivated silica gel, tapping firmly to settle, then adding 2.5 cm of Na_2SO_4, and tapping again. Prewash with ten ml of hexane and when the hexane wash just reaches the top of the Na_2SO_4, place a 13-ml centrifuge tube under the column to receive the first fraction. Add 0.5 ml of hexane to the tube containing the sample, mix on the Vortex mixer, and add the sample to the top of the bed with a disposable pipet. When this portion has sunk into the bed, repeat with three more 0.5-ml portions of hexane. Finally, add eight ml of hexane to the tube, mix, and add to the top of the bed. All subsequent eluents are measured in the original sample tube and added to the bed with the same disposable pipet. After the first ten-ml hexane fraction has been collected, place a 15-ml centrifuge tube under the column and add 15 ml of 60% benzene in hexane to the column in portions of 5 and 10 ml. After this second 15 ml fraction is collected, elute the column with 15 ml of 5% acetonitrile in benzene into a 15-ml centrifuge tube.

Figure 1 outlines this procedure and lists the pesticides studied (Table I) and their elution pattern from the silica gel column. Fraction I contains six chlorinated compounds, Fraction II contains five phosphate and five chlorinated compounds, and Fraction III contains two phosphates and all seven carbamates.

Determination of pesticides in the fractions. **Fraction I**: Evaporate Fraction I to 2.5 ml under a stream of nitrogen and inject five μl onto the 5% OV-210 column (EC detector). Relative retention times for pesticides in this fraction are listed in the Table II. Occasionally, lindane was found to split between Fractions I and II. This presents no problem since lindane is resolved and can be quantitated in both fractions. Figure 2 shows a

typical chromatogram of the first fraction, a chromatogram of a reagent blank run through the entire procedure, and the amounts of each pesticide injected for the low-level recovery studies.

Fraction II: Evaporate Fraction II to 2.5 ml under a stream of nitrogen. Inject five μl onto the 5% OV-210 column (EC detector). All pesticides in this fraction are resolved except dieldrin and methyl parathion (Table III). This fraction is further concentrated to 1.0 ml and 20 μl are injected onto the Carbowax-treated OV-210 column (FPD detector) to determine the phosphate compounds. If methyl parathion is present, dieldrin is quantitated by injection of a portion of Fraction II onto the 5% SE-30 column (EC detector). Dieldrin is well separated from methyl parathion and all other compounds (Table III).

Figure 3 shows the chromatogram of Fraction II and a reagent blank taken through the whole procedure on the OV-210 column (EC detector). A large background peak elutes just after heptachlor epoxide with approximately the same retention time as *p,p'*-DDE. This pesticide, however, elutes from silica gel in the first fraction, and only a small interfering peak in this position was noted in Fraction I. Figure 4 shows Fraction II on the OV-210 column (FPD). A small background peak is found with the same retention

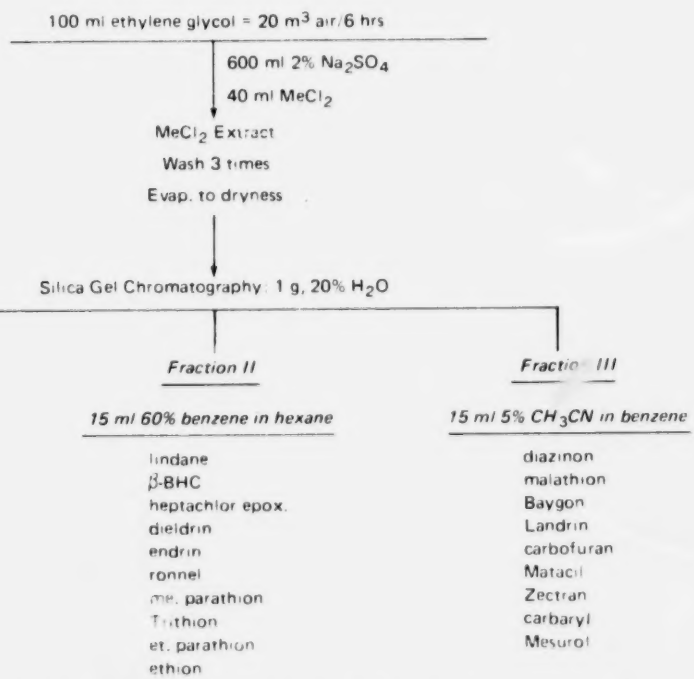

Fig. 1. New procedure for detection of pesticides in ethylene glycol.

Table I. *Chemical designations of pesticides studied*

Common or trade name	Chemical designation
aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene
Baygon®	*o*-isopropoxyphenylmethylcarbamate
α-BHC	α-isomer of 1,2,3,4,5,6-hexachlorocyclohexane
β-BHC	β-isomer of 1,2,3,4,5,6-hexachlorocyclohexane
carbaryl	1-naphthyl *N*-methylcarbamate
carbofuran	2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate
p,p'-DDD	1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane
p,p'-DDE	1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethylene
o,p'-DDT	1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane
p,p'-DDT	1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl) ethane
diazinon	*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate
dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo,exo*-5,8-dimethanonaphthalene
endrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4-4a,5,6,7,8,8a-octahydro-1,4-*endo,endo*-5,8-dimethanonaphthalene
ethion	*O,O,O',O'*-tetraethyl *S,S'*-methylenebis-phosphorodithioate
heptachlor epoxide	1,4,5,6,7,8,8a-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindene
2,3,5-Landrin®	2,3,5-trimethylphenylmethylcarbamate
lindane	γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane
malathion	*O,O*-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithionate
Matacil®	4-dimethylamino-*m*-tolyl methylcarbamate
Mesurol®	4-(methylthio)-3,5-xylyl methylcarbamate
parathion, ethyl	*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate

Table I *(Continued)*

Common or trade name	Chemical designation
parathion, methyl	*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate
ronnel	*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate
Trithion	*O,O*-diethyl S-[(*p*-chlorophenylthio)methyl] phosphorodithioate
Zectran®	4-dimethylamino-3,5-xylyl *N*-methylcarbamate

Table II. *Analysis of fraction I*[a]

Compound	Relative retention time[b]
α-BHC	0.64
lindane	0.81
aldrin	1.00
p,p'-DDE	2.10
o,p'-DDT	2.70
p,p'-DDD	3.75
p,p'-DDT	4.07

[a] 5% OV-210 column, 180°C, 60 ml/min carrier gas flow rate, EC detector, 10 × 16 electrometer setting, 5 μl injected from 2.5 ml sample volume.
[b] Relative to aldrin

time as ethion. Figure 5 shows Fraction II on the SE-30 column (EC detector) only in the region of the dieldrin peak.

Fraction III. Evaporate Fraction III to 0.5 ml and inject ten μl on the OV-210 column under the conditions shown in Table III for the FPD detector. Diazinon and malathion are selectively detected and well resolved (retention times 0.75 and 2.91 relative to aldrin, respectively). The fraction is then evaporated just to dryness (keeper) under a stream of nitrogen and the carbamates derivatized with pentafluoropropionic anhydride (PFP) by the method of Shafik *et al.* (1972). The carbamates are analyzed on the 5% SE-30 column under the conditions specified earlier.

Figure 6 shows the chromatogram and blank for Fraction III on the OV-210 column. Two large background peaks are found between the two pesticide peaks but are resolved from them.

The procedure (Shafik *et al.* 1972) for derivatization of the carbamates is as follows: After evaporation to dryness add two ml of isooctane, one drop of redistilled pyridine, and 0.025 ml of PFP. Mix well and allow the reaction to proceed for one hr at room temperature. Add three ml of phosphate buffer and mix on a Vortex mixer to stop the reaction. Add three ml of isooctane, 0.05 ml of acetonitrile, and mix again for 30 sec. After separation of the layers, remove the bottom layer with a disposable pipet and discard. Add two ml of water, mix, and again aspirate bottom layer. Repeat the washing two more times. Centrifuge and remove the last water droplets collected at the bottom of the tube. Add about 0.5 g of sodium sulfate, mix, and inject five μl for analysis.

Figure 7 shows the separation of seven carbamate insecticides in Fraction III and the reagent blank. Background peaks are noted under Baygon and carbaryl, and very large peaks under Matacil and Zectran. Other background peaks are separated from the pesticides. The background peaks preceding Matacil and Mesurol are due to reagents used in the derivatization procedure. The others arise from the ethylene glycol.

Diazinon, if present in Fraction III, would co-elute from the SE-30 column with carbaryl under the conditions used. The response for diazinon under these conditions is

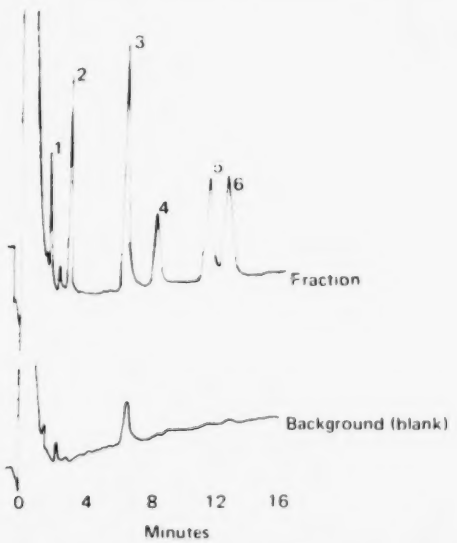

Fig. 2. Chromatograms of Fraction I and a blank obtained under the conditions shown in Table II. 1 = α-BHC, 4 pg, 2 = aldrin, 10 pg, 3 = *p,p'*-DDE, 20 pg, 4 = *o,p'*-DDT, 30 pg, 5 = *p,p'*-DDD, 40 pg, 6 = *p,p'*-DDT, 20 pg.

quite low, so that amounts < two ng will not interfere. If large amounts of diazinon are present, Fraction III must be further treated to quantitate carbaryl accurately. Transfer 1.5 ml of the solution of the derivatized carbamates to a 13-ml centrifuge tube, add one ml of 6*N* NCl, and vortex mix for one min. Allow to stand for five min and mix again for one min. Allow the layers to separate, remove the bottom layer, wash the isooctane with 2 × 2 ml of water discarding the water each time, add about 0.5 g of Na_2SO_4, and inject five μl for analysis. Diazinon, Matacil and Zectran are removed as water-soluble hydrochorides by the acid treatment. Carbaryl is confirmed and quantitated from the chromatogram of the acid-treated fraction. The heights of the background peaks remaining after the removel of Matacil and Zectran are subtracted from the peak heights found in the original Fraction III for quantitation of these two carbamates.

Table III. *Analysis of fraction II*

Compound	Relative retention time[a]	
	OV-210[b]	SE-30[c]
lindane	0.81	—
β-BHC	0.96	—
ronnel	1.36	—
heptachlor epoxide	1.93	1.21
dieldrin	2.95	1.77
methyl parathion	2.95	0.69
endrin	3.61	1.98
ethyl parathion	3.87	—
Trithion	4.87	—
ethion	5.37	—

[a] Relative to aldrin

[b] Chlorinated compounds quantitated on 5% OV-210 column, 180°C, 60 ml/min carrier gas flow rate, EC detector, 10 × 8 electrometer setting, 5 μl injected from 2.5 ml sample volume.

Phosphates quantitated on 5% OV-210 Carbowaxed column, 180°C, 60 ml/min carrier gas flow rate, FPD detector (P mode), 10^3 × 16 electrometer setting, 20 μl injected from 1.0 ml sample.

[c] If dieldrin and methyl parathion are both present, quantitate dieldrin on 5% SE-30 column, 195°C, 70 ml/min carrier gas flow rate, EC detector, 10 × 8 electrometer setting, 5 μl injected from 2.5 ml sample.

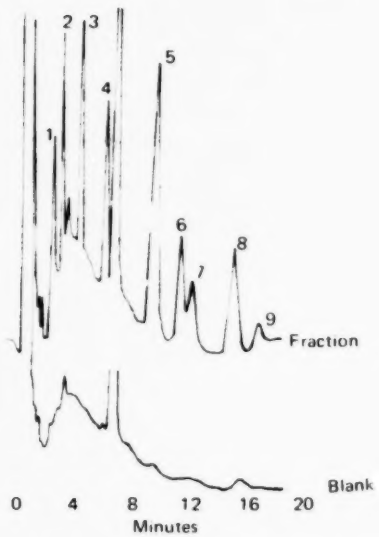

Fig. 3. Chromatogram of all pesticides in fraction II and a blank obtained on a 5% OV-210 column, EC detector, under conditions shown in Table III. 1 = lindane, 4 pg, 2 = β-BHC, 2 pg, 3 = ronnel, 16 pg, 4 = heptachlor epoxide, 10 pg; 5 = dieldrin, 20 pg + methyl parathion, 32 pg, 6 = endrin, 20 pg, 7 = parathion, 40 pg, 8 = trithion, 80 pg, 9 = ethion, 46 pg.

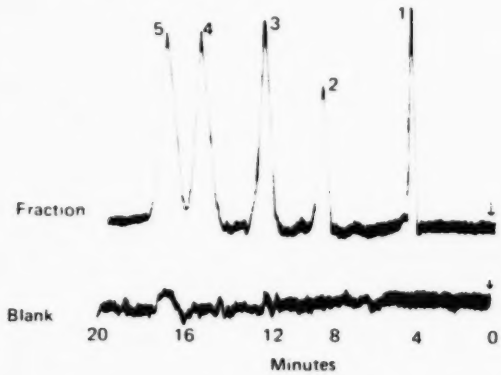

Fig. 4. Chromatogram of phosphate pesticides in fraction II and a blank on the 5% OV-210 column, FPD detector, under conditions shown in Table III. 1 = ronnel, 160 pg, 2 = methyl parathion, 320 pg, 3 = ethyl parathion, 400 pg; 4 = Trithion, 800 pg; 5 = ethion, 460 pg.

Figure 8 shows a chromatogram of a mixture of carbamate standards after acid treatment and clearly indicates the efficiency of removal of the two pesticide peaks. Also shown is Fraction III after acid treatment with the background peaks left after removal of Maracil and Zectran (peaks 4 and 5). Finally, a reagent blank run through the entire

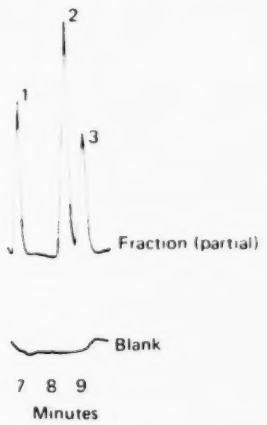

Fig. 5. Partial chromatogram illustrating the separation of dieldrin in fraction II and a blank on the 5% SE-30 column under conditions shown in Table III. 1 = heptachlor epoxide, 10 pg; 2 = dieldrin, 20 pg; 3 = endrin, 20 pg.

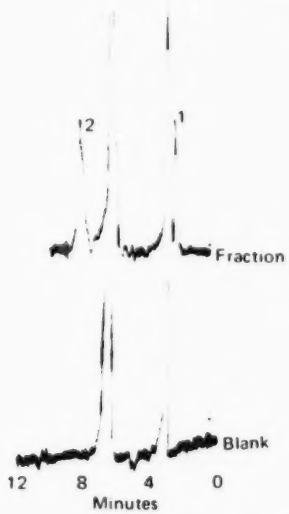

Fig. 6. Chromatogram of phosphate pesticides in fraction III and a blank on the 5% OV-210 column under conditions described in the text. 1 = diazinon, 80 pg; 2 = malathion, 400 pg.

procedure and acid treated also retains the same two background peaks (4 and 5). Malathion present in Fraction III is decomposed by the derivatization procedure.

Results and discussion

Table IV shows recovery data for 25 compounds from ethylene glycol spiked at the high and low levels studied and carried through the entire procedure. Quantitation was based on peak heights compared with standards injected directly before and after each sample. Recoveries were between 75% and 109% for all compounds except Baygon, ethion, Matacil and Zectran at the low levels. Recoveries of the first two of these pesticides were high because of the interfering background peaks shown in Figures 4 and 7. Matacil and Zectran showed low recoveries because the background subtraction procedure just described may not accurately correct for the interfering background peaks, although Figure 8 indicates that in principle it should. Runs made at the higher levels (Table IV) gave better results for these pesticides. Problems other than the subtraction procedure may be involved in these poor low level recoveries. The low spike levels shown in Table IV correspond to the required pesticide quantitation levels of 1-4 ng/m^3 of air in the National Air Monitoring Progeam (Enos *et al.* 1972) and represent the approximate lower limits of the present procedure because of background peaks from the ethylene glycol. The method described for cleanup of ethylene glycol was the best of several we tried. Although we routinely used redistilled ethylene glycol, one test made with crude ethylene glycol cleaned up by the same procedure showed essentially no difference.

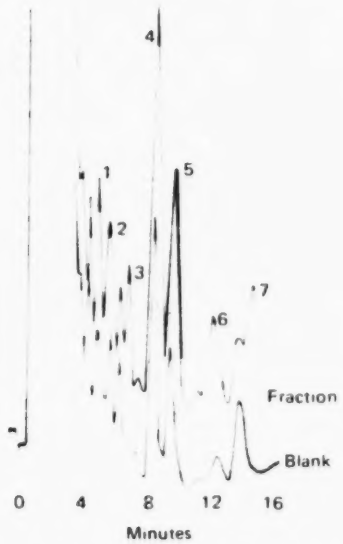

Fig. 7. Chromatogram of PFP derivatives of *N*-methyl carbamate pesticides in fraction III and a blank on the 5% SE-30 column under the conditions described in the text. 1 = Baygon, 20 pg, 2 = 2,3,5-Landrin, 40 pg, 3 = carbofuran, 40 pg, 4 = Matacil, 80 pg, 5 = Zectran, 80 pg, 6 = carbaryl, 40 pg, 7 = Mesurol, 40 pg

Although only a limited number of important pesticides were included in the present study for the purposes of developing the procedure, it is felt that the method devised has important advantages over the present one (Thompson 1971) with the potential for determining more phosphate compounds in addition to carbamates. It is known, for example, that hexane does not extract certain phosphate and carbamate pesticides. Also, many phosphate and carbamate compounds are not successfully eluted from Florisil columns. Another advantage of the new procedure is that it is a micro rather than a macro method. After the initial single extraction with methylene chloride in a one-liter separatory funnel, convenient, small-scale equipment is used throughout.

Many other adsorbent systems were tested before choosing silica gel deactivated with 20% water and the eluents described above. Silica gel eluted with cyclohexane-benzene mixtures or charcoal mixture (Storherr *et al.* 1971) and 5% water-deactivated acidic, basic and neutral alumina eluted with hexane-benzene mixtures did not separate the chlorinated and phosphate compounds from each other as well as the system finally chosen. Silica gel with 20% water has advantages over silica gel deactivated with lower amounts of water in that the activity is easier to reproduce and smaller volumes and less-polar solvents are required for elution of the polar pesticides. It has been reported (Berg *et al.* 1972) that the reproduction of adsorbent activity is difficult, but we had no problem in reproducing elution results from day to day. We feel that fewer problems can be anticipated using silica gel deactivated with 20% water.

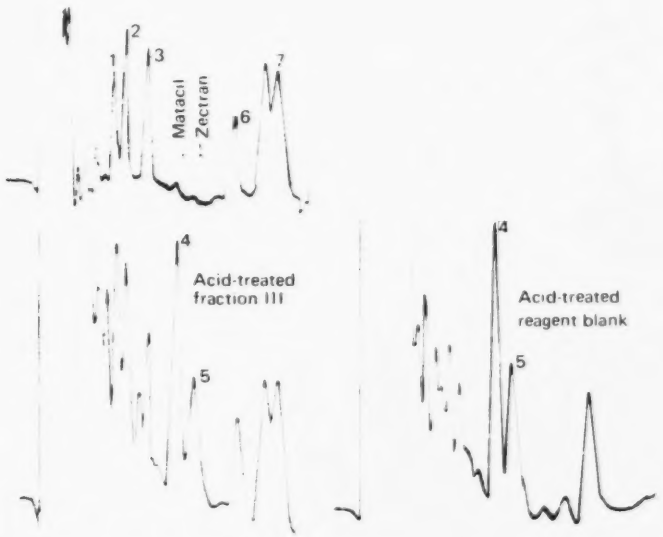

Fig. 8. Chromatograms on 5% SE-30 column under conditions described in the text of acid-treated carbamate standards, acid-treated fraction III, and an acid-treated reagent blank. Peaks numbered 4 and 5 are background peaks remaining after removal of Matacil and Zectran. Other numbers correspond to Fig. 7.

Although the 5% OV-210 column was initially chosen and used throughout this work for quantitation of the chlorinated compounds in Fraction II, the 5% SE-30 column operated at 195°C and 70 ml/min nitrogen carrier gas flow rate is an attractive alternative, especially if it is necessary to quantitate all chlorinated and phosphate compounds in Fraction II using the electron capture detector. All ten compounds eluting in Fraction II are at least partially separated on the SE-30 column in the following sequence:

Table IV. *Recoveries of pesticides at low and high levels from 100 ml of ethylene glycol*

Pesticide	Low level		High level	
	Spike (ng)	Recovery (%)[a]	Spike (ng)	Recovery (%)[a]
α-BHC	2	87	6.4	80
aldrin	5	95	8	77
p,p'-DDE	10.	106	16	99
o,p'-DDT	15.	100	48	92
p,p'-DDD	20.	103	64	84
p,p'-DDT	10.	109	64	95
lindane	2	92	8	85
β-BHC	1	91	8	80
hept. epox.	5	93	18	87
dieldrin	10	101	40	92
endrin	10	95	80	92
ronnel	20	96	84	81
parathion (me)	40	97	162	87
parathion (et)	50	93	207	90
Trithion	100	107	376	81
ethion	60	129	246	97
diazinon	10	97	109	92
malathion	50	104	460	97
Baygon	20	126	1000	86
2,3,5-Landrin	20	81	1000	95
carbofuran	40	75	2000	86
Matacil	80	59	4000	78
Zectran	80	52	4000	81
carbaryl	40	103	2000	104
Mesurol	40	96	2000	95

[a] Average of 3 determinations for each pesticide.

Pesticide	*Retention relative to aldrin*
β-BHC	0.45
lindane	0.50
(large interfering peak)	(0.60)
methyl parathion	0.69
ronnel	0.78
parathion	0.94
heptachlor epoxide	1.21
dieldrin	1.77
endrin	1.98
ethion	2.35
trithion	2.65

The early eluting compounds are not as well resolved on this column as on the OV-210 column, but quantitation of all compounds is possible without re-injection on a second column. The OV-210 and SE-30 columns used together are useful for confirmation of peak identities because of the markedly different elution patterns obtained. Diazinon and malathion in Fraction III cannot be quantitated using the electron-capture detector because of an extremely high background which masks both pesticide peaks.

Several attempts were made to isolate Matacil and Zectran from the background interferences to quantitate these pesticides directly rather than by subtraction as described. Treatment of Fraction III with coagulating mixture before derivatization removed the Matacil and Zectran peaks, but adjustment of the aqueous phase to pH 7, followed by extraction with $MeCl_2$ and derivatization, did not recover the compounds. Adjustment to pH 9 led to partial recovery of the compounds but also gave a very high background. Adjustment of the aqueous acid phase to pH 8 after treatment of derivatized Fraction III with 6*N* HCl, followed by extraction with $MeCl_2$ and rederivatization, gave no recovery of the two pesticides. In view of these unsuccessful experiments and the inability to find an alternate GC column that would separate the pesticide peaks from the background, the best approach available appeared to be subtraction of background remaining after acid treatment from the original peaks. Another possibility that may be superior for the analysis of the carbamates in this scheme is hydrolysis to the carbamate phenols followed by derivatization (Sullivan and Shafik 1973; Seiber 1972).

The pre-filter cloth used in the air-sampling train was tested for background by washing with hexane and acetone and then soaking in $MeCl_2$ overnight. The $MeCl_2$ was evaporated to a small volume and a portion injected in the electron capture – GC with and without derivatization with PFP. The background was essentially nil without derivatization, but extremely high after derivatization, indicating that this material was unsatisfactory if carbamates were to be analyzed using the PFP procedure. A Teflon mesh material was obtained from Ace Gasket Inc., Mt. Vernon, New York, and similarly tested. In this case, the background on the SE-30 GC column consisted of several early peaks and three low peaks in the area where the carbamates would elute. It appeared that this Teflon material would be quite satisfactory for use as a pre-filter after soaking in $MeCl_2$.

The ability of the Greenburg-Smith type impingers charged with ethylene glycol to efficiently trap and concentrate chlorinated and phosphate pesticides from ambient air and the stability of the pesticides in this collection system has been well documented (Enos *et al.* 1972; Miles *et al.* 1970). The stability of carbamates in the collection system was checked by spiking 100 ml of ethylene glycol in an impinger with the seven insecticides at levels corresponding to 5 to 20 ng/m^3 and drawing air through for six hr at a rate of one cu ft per min and analyzing the ethylene glycol. Recoveries were 73% for Matacil (corrected for background) and 84% or more for the other six compounds. The trapping efficiency for carbamates has not been determined.

It is expected that the general approach described in this report would successfully determine multiresidues of pesticides in samples other than air with perhaps some additional cleanup steps (e.g., gel permeation chromatography) for especially difficult samples such as human fat. With this in mind, and since acetonitrile is a common solvent for extracting pesticides from food, fat, blood and other types of samples, the partitioning of the chlorinated and phosphate pesticides between acetonitrile and $MeCl_2$ was studied. It was determined that all 18 compounds were extracted with $> 90\%$ recovery by $MeCl_2$ when the ratio of four ml of acetonitrile to 25 ml 2% Na_2SO_4 was used and extraction was made with five ml of $MeCl_2$ followed by two more two-ml portions. The complete extraction of the seven carbamate insecticides by $MeCl_2$ under these conditions has already been reported (Shafik *et al.* 1972).

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Manuscript received January 21, 1974; accepted May 8, 1974

PB 275 985

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Applications of Combined Gas Chromatography–Mass Spectrometry to Pesticide Residue Identifications

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Several individual and multiresidue analytical methods are available for gas chromatographic–mass spectrometric confirmation and identification of pesticide residues. Specific examples considered in this report include the analysis of intact and derivatized phenolic residues such as pentachlorophenol and 1-naphthyl chloroacetate, organophosphorus insecticide metabolic and hydrolytic products including O,O-diethyl O-methyl phosphorothionate, O,O-diethyl S-methyl phosphorothiolate, and O,O-diethyl O-methyl phosphate, several organochlorine pesticides of the DDT and cyclodiene type, phenoxyalkanoic acid herbicide exposure and methodology studies involving 2,4-D and 2,4,5-T, and polychlorinated biphenyl residues. Conventional residue analytical methodology and gas chromatographic column technology are, in general, directly applicable to the analysis of human, animal, and environmental substrates by the combined technique. Evaluation of mass spectral fragmentation pathways provides definitive and conclusive confirmation of residue identity as well as characterization of residues and their metabolites of unknown structure.

Unequivocal identification of pesticides, pesticide metabolites, and other chemical environmental pollutants in most cases requires more evidence than can be provided by a single chromatographic method. Several approaches may be utilized to secure firm proof of identity. For example, supportive chromatographic data such as relative retention

values on two gas chromatographic columns of differing characteristics may be obtained. Of further value is information derived by means of thin-layer chromatography (R_f values), the solvent partitioning characteristics of the residue of interest (p-values), and the response behavior on several more or less specific gas chromatographic detectors. In addition, spectrometric techniques such as infrared and ultraviolet spectrophotometry may be applied to provide even less ambiguous proof of residue structural identity. The value of mass spectrometry as a tool for providing the structural identity of complex organic molecules has become well recognized in recent years. Mass spectra furnish information concerning the structural arrangement of atoms within a molecule on the basis of the mode of fragmentation of the compound as a radical ion, usually produced by electron bombardment. Fragmentation patterns resulting from bond fission and rearrangement of atoms are highly diagnostic and characteristic of the original molecular structure. Interpretations are usually made on the basis of empirical correlations, comparison with the mass spectra of standard materials, or detailed studies of ion decomposition mechanisms. The theories and principles governing the interpretation of mass spectra have been discussed in many reference texts and comprehensive reviews (*1*, *2*).

The potential utility of mass spectrometry in evaluating the metabolic pathways of pesticidal chemicals by providing the structural identity of metabolites was stated as early as 1962 in a review by Gunther (*3*). More recently, several reviews (*4*, *5*, *6*) have considered the role of mass spectrometry in chemical structure evaluations with special reference to pesticide residue analysis.

Correlations of mass spectral fragmentation pathways with the structure of pesticides are required to obtain background information useful in the interpretation of the mass spectra of unknown pesticide metabolites and other conversion products. Fortunately, the chemical modes of fragmentation of a large number of pesticidal compounds have been determined (*7*). This information is particularly useful in those instances when only microgram quantities of materials are available for characterization; mass spectrometry offers one of the best instrumental approaches in view of the low sensitivity of such complementary techniques as nuclear magnetic resonance spectrometry. The investigator may then rely on this single source of information for all the structural data. An additional advantage of the mass spectrometric approach is the availability of combined gas chromatograph–mass spectrometer instruments which permit high sensitivity analyses of multicomponent mixtures. Mass spectrometry, of course, has been widely employed as an individual technique for analysis of residues and metabolites isolated by conventional separation techniques such as thin-layer, liquid–paper, gas–liquid, and

column chromatography. In these instances, specialized techniques are employed for the efficient trapping and collection of individual gas chromatographic peaks or thin-layer chromatographic spots for subsequent mass spectral analysis by standard sample vacuum introduction methods.

Recent reports have focused attention on the need for confirming pesticide residues, particularly those isolated from environmental samples (8, 9), and for chemical identity in ultramicroanalysis in general. Of the spectrometric techniques available for confirmation of residues, the combined gas chromatographic–mass spectrometric approach affords many advantages including relatively high sensitivity, elimination of the necessity for isolating minute quantities of pure samples with standard chromatographic methods, and a greater certainty in identification of an eluted component than that achieved by conventional detector response characteristics and gas chromatographic retention times alone. Because of the definitive structural information obtained by mass spectrometry, proper use of the technique would obviate the necessity for the application of two or more different carefully selected techniques for characterizing pesticide residues (8).

An additional obvious advantage would be the characterization and identification of frequently encountered, unknown components of extracts, which may represent pesticidal metabolites, photochemical and other "weathered" residues, nonpesticidal chemical pollutants, or co-extractive interferences whose identity may be required.

This report presents a discussion of recent applications of combined gas chromatography–mass spectrometry to analysis of pesticide residues isolated from human, animal, and environmental media. Emphasis will be placed on analytical techniques, related gas chromatographic column technology, and analysis of fragmentation pathways pertinent to the identification of pesticide residues.

Experimental

The mass spectrometric analyses initially reported in this communication were performed with a double focusing low resolution mass spectrometer, Model 270, Perkin-Elmer Corp., Norwalk, Conn., coupled through a Watson–Biemann type of molecular separator with a gas chromatographic system. (Commercial sources and trade names are provided for identification only. Their mention does not constitute endorsement by the Public Health Service or by the U. S. Dept. of Health, Education and Welfare.)

Coiled glass gas chromatographic columns were employed except where otherwise indicated. Programmed temperature analyses were made with the initial and final oven conditions as well as the program rate given in the discussion. The molecular separator and gas inlet temperatures were maintained at 200° and 210° C, respectively. All mass spectra were

recorded at 80 eV electron energy with 2400 V accelerating voltage; the filament emission current was 100 μa. Chromatograms were recorded from the total ion current monitor located between the electrostatic and magnetic analyzer sectors. Helium carrier gas was approximately 10 ml per min for the packed chromatographic columns and approximately 2 ml per min for the capillary columns. Injector temperature was maintained at 165 C. Mass spectra were scanned magnetically over the range of interest at a rate of either 3 or 10 sec per decade. The combined gas chromatograph–mass spectrometer system has been described in more detail elsewhere (*10*).

Phenolic Residues

Confirmation of the identity of pentachlorophenol (PCP) in samples of human blood, urine, tissue, clothing, and bedding materials has been reported (*11*), utilizing combined gas chromatographic–mass spectrometric analysis of hexane extracts of these substrates.

This report constituted a detailed account of the analytical aspects of an epidemic of infant deaths associated with the ingestion of this compound, which was the active ingredient of a mildew preventative, the suspected source of exposure. Analysis of the hexane extracts was performed on a 1.0 m × 2.5 mm coiled glass gas chromatographic column packed with 3% DEGS and 2% concentrated phosphoric acid. Under these conditions, analysis could be performed without prior derivatization of the PCP. All sample spectra gave four identical major ions when compared with PCP standard mass spectrum obtained at 20 eV. Thus, identity of the residues of PCP was confirmed on the basis of the observation of the molecular ion peak at m/e 264, a fragment ion of m/e 229, owing to elimination of a Cl atom, a major fragment resulting from the consecutive loss of HCl and CO at m/e 200, and finally a characteristic fragment of

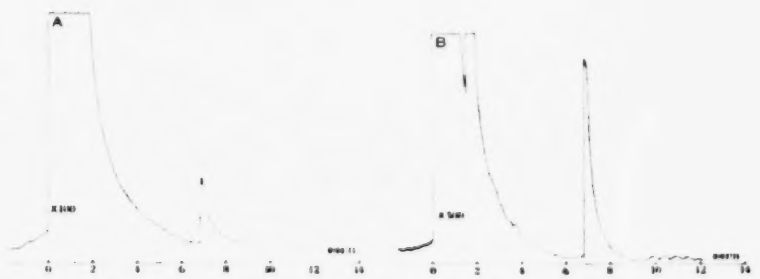

Figure 1. Total ion current chromatograms of (A) standard 1-naphthyl chloroacetate (1 μg) and (B) 1-naphthyl chloroacetate isolated from human urine
Programmed temperature conditions: two minutes at 165 C, to 185 C at 5 C/min, isothermal at 185 C

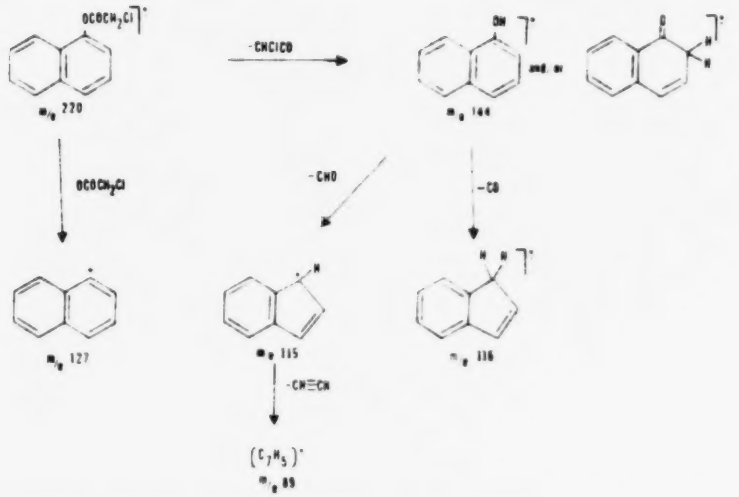

Figure 2. Electron impact-induced fragmentation scheme for 1-naphthyl chloroacetate

m/e 165 undoubtedly resulting from loss of a chlorine atom from the m/e 200 ion.

During the course of analytical method studies involving the development of procedures for the detection and quantitation of 1-naphthol, a major metabolite of carbaryl, in human and animal urine (*12*), the derivatized residue, 1-naphthyl chloroacetate, was confirmed by gas chromatography–mass spectrometry. The analytical procedure for the isolation of 1-naphthol residues involved acid hydrolysis of 1-naphthol conjugates in the urine, benzene extraction, derivatization with chloroacetic anhydride and pyridine, and removal of interfering material in urine by column chromatography using silica gel. 1-Naphthyl chloroacetate was eluted with 60% benzene–hexane. Programmed temperature gas chromatographic analysis with a coiled glass column, 4 ft × 1/8 inch o.d., packed with 2% SE-30 on 60/80 mesh Gas Chrom Q, was used to confirm residues of 1-naphthol as the chloroacetate ester in human urine of individuals occupationally exposed to carbaryl (Figure 1). Diagnostic mass spectral peaks observed for 1-naphthyl chloroacetate included the molecular ion of m/e 220, a base peak fragment which may be formulated as a 1-naphthol radical ion (or an isomer) found at m/e 144 arising from hydrogen rearrangement and loss of —CHClCO, a relatively weak peak at m/e 127 formed either by elimination of —OCOCH$_2$Cl from the molecular ion and/or by loss of water from the m/e 145 fragment, and finally, other peaks characteristic of the fragmentation of 1-naphthol at m/e 115

and m e 116 arising from expulsion of CHO and CO, respectively, and a peak at m e 89 presumably resulting from a $C_7H_5^+$ ion formed by the loss of acetylene (C_2H_2) from the m e 115 fragment (Figure 2).

Organophosphorus Pesticides

In connection with the development of an analytical method (*13*) for the determination of organophosphorus pesticides in human blood and urine, mass spectral confirmation of a series of methylated and ethylated derivatives of the hydrolytic and metabolic products of these insecticides was required. The urine of an individual occupationally exposed to parathion was extracted with a 1:1 (v v) solvent mixture of acetonitrile and diethyl ether. Simultaneously, the intact organophosphorus insecticides were hydrolyzed by adding a portion of 5*N* hydrochloric acid to

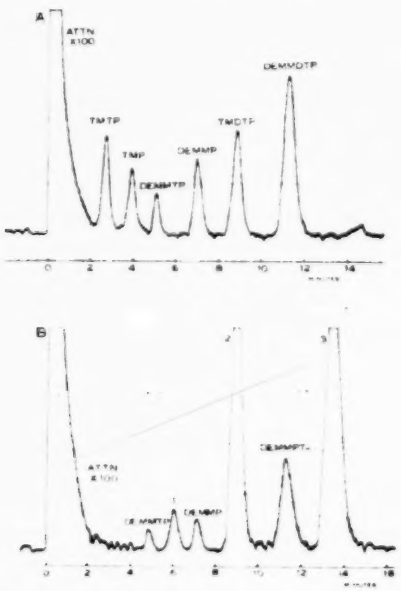

Figure 3. Total ion current chromatograms of (A) standard mixture of methylated dialkylphosphates, phosphorothioates, and phosphorodithioates (2 μg) and (B) human urine extract containing methylated hydrolytic and metabolic products of parathion

Programmed temperature conditions: five minutes at 75 C to 120 C at 5 C min isothermal at 120 C

the urine. Following extraction, the organic layer was concentrated and the residues in solution derivatized by means of diazomethane. The urine extract was then subjected to a clean up procedure employing silica gel column chromatography (*14*), and benzene (I), 40% ethyl acetate in benzene (II), and 80% ethyl acetate in benzene (III) as eluting solvents. Concentration of the chromatographic column eluents provided the urine extract which was subjected to programmed temperature analyses on a coiled glass capillary column, 110 ft × 0.025 inch i.d., coated with Versamid 900 containing 5% Igepal CO-880. The resulting total ion current chromatogram is shown in Figure 3. Also illustrated is the total ion current trace obtained by analysis of a series of methylated dialkyl phosphate standard materials.

The metabolic and or hydrolytic products of parathion encountered as residues in the urine include both diethyl phosphoric acid and diethyl phosphorothioic acid, most probably as their salts (potassium or sodium). Derivatization of these residues with diazomethane would result in the formation of three trialkyl phosphate compounds, namely, *O,O*-diethyl *O*-methyl phosphate (DEMMP), *O,O*-diethyl *O*-methyl phosphorothionate (DEMMTP), and *O,O*-diethyl *S*-methyl phosphorothiolate (DEMMPTh). Earlier (*15*), it had been shown by combined gas chromatography–mass spectrometry and other analytical data that a later-eluting major product (*ca.* 85%) of the methylation of diethyl phosphorothioic acid formed under the conditions of the analytical method was DEMMPTh, and the minor product formed (*ca.* 15%) was DEMMTP. Accordingly, all three trialkyl phosphates were observed and confirmed by mass spectrometry in the analysis of the human urine extract. Sufficient internal bond energy differences are associated with the isomeric structures DEMMPTh and DEMMTP that qualitatively and quantitatively dissimilar fragmentation patterns are observed for both isomers as can be seen from the mass spectra of these compounds shown in Figure 4.

The mass spectrum of DEMMTP is characterized by both phosphorus–oxygen and carbon–oxygen bond fission as evidenced by the formation of fragment ions at m/e 140 ($M-C_2H_4O$) and m/e 156 ($M-C_2H_4$). Single and double hydrogen rearrangements accompany the loss of the ethyl substituent. Subsequent fragmentation reactions noted were similar bond fission involving elimination of the remaining alkyl substituents as an ethylene molecule or an ethoxyl radical. A relatively intense peak, for example, of m/e 111 [$CH_3OP(S)-OH$], appears to be formed by consecutive loss of ethylene (to form the m/e 156 ion) and an ethoxyl substituent from the molecular ion as evidenced by metastable peaks at m/e 132.26 and m/e 78.98 observed for these two processes. Significant fragment ions were also formed through loss of both sulfur atoms or sulfhydryl

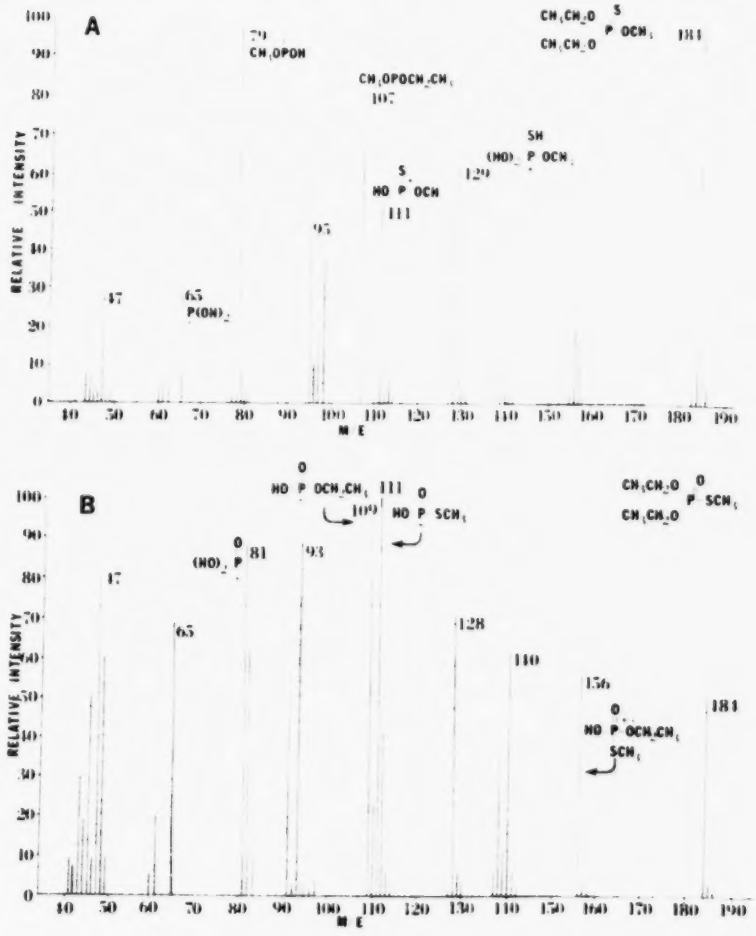

Figure 4. Mass spectra of (A) O,O-diethyl O-methyl phosphorothionate (DEMMTP), and (B) O,O-diethyl S-methyl phosphorothiolate (DEMMPTh)

radicals from intermediate daughter ions. Figure 5 illustrates the major dissociative processes observed in the mass spectrum of DEMMTP. The P(=S)–OR to P(=O)–SR isomerization induced under electron impact conditions for compounds of this structure could not be confirmed for DEMMTP although other investigators have observed this rearrangement for organic phosphorothionates (*16, 17*). Other trialkyl phosphorothionate

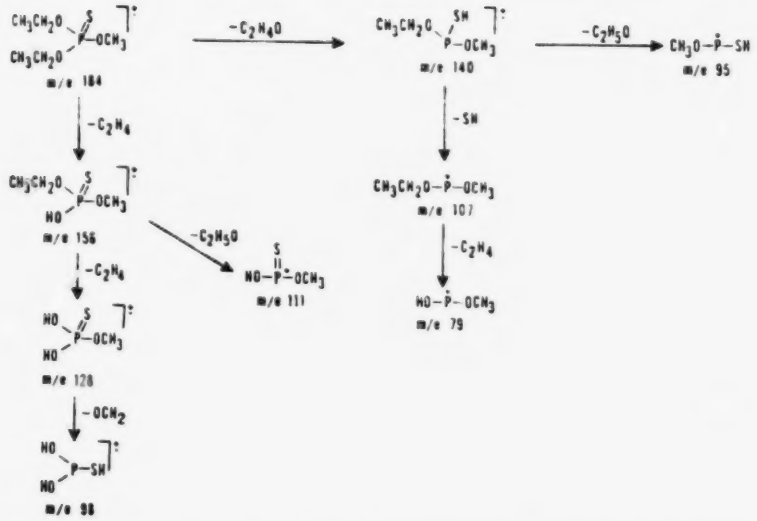

Figure 5. Electron impact-induced fragmentation scheme for O,O-diethyl O-methyl phosphorothionate (DEMMTP)

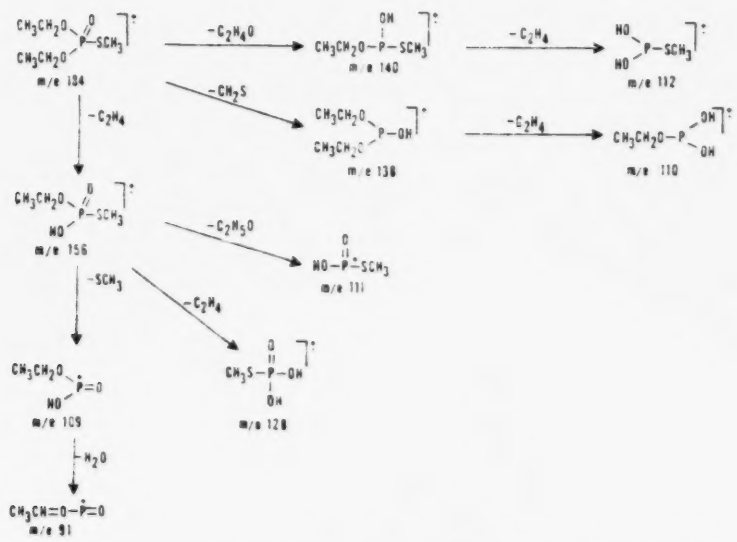

Figure 6. Electron impact-induced fragmentation scheme for O,O-diethyl S-methyl phosphorothiolate (DEMMPTh)

compounds have also been observed to undergo this rearrangement process (*18*).

A somewhat more complex dissociative scheme was observed for DEMMPTh (Figure 6). The molecular ion of this compound decomposes by fission of a carbon–oxygen bond (m/e 156, M–C_2H_4), phosphorus–oxygen bond (m/e 140, M–C_2H_4O), and the phosphorus–sulfur bond (m/e 138, M–CH_2S). The structure of the m/e 156 fragment (Figure 6) permits a subsequent identical triple decomposition scheme to yield fragments of m/e 128 (156–C_2H_4), m/e 109 (156–CH_3S), and m/e 111 (156–C_2H_5O). Another significant reaction observed was the elimination of water which, for example, led to the formation of an intense ion fragment at m/e 91 (109–H_2O), characteristic of the electron impact fragmentation of esters

The decomposition of DEMMP under electron impact results in the formation of significant fragment ions of m/e 141, m/e 113, and m/e 95, according to the dissociative scheme illustrated in Figure 7.

Organochlorine Pesticides

Chlorinated hydrocarbon pesticide residues in human adipose tissue and liver tissue samples have been identified by mass spectrometry coupled with gas chromatography. A general, extensive extraction and cleanup procedure adapted from existing methods was used to isolate

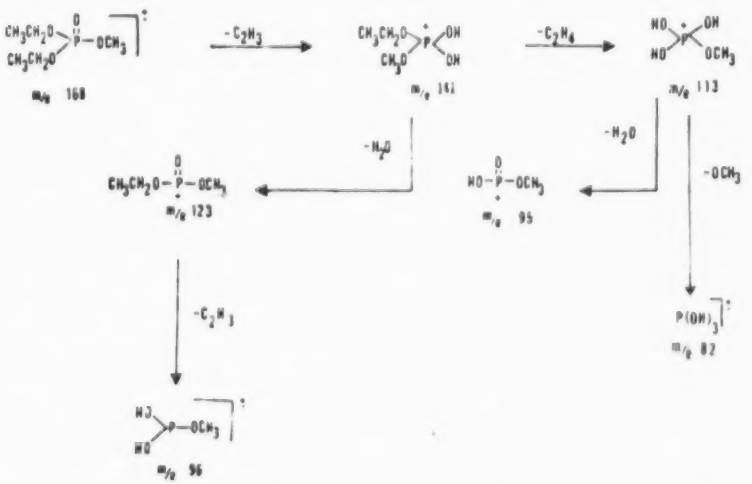

Figure 7. Electron impact-induced fragmentation scheme for O,O-diethyl O-methyl phosphate (DEMMP)

and purify the organochlorine residues. The tissue samples were extracted with acetonitrile or petroleum ether, followed by liquid–liquid partitioning of the extract between hexane or petroleum ether and acetonitrile. Column chromatography and cleanup on Florisil provided two individual fractions (*19*), which were then concentrated and subjected to further cleanup and pesticide residue fractionation by means of silica gel column chromatography employing a published procedure (*20*). The pesticide residues were separated by the silica gel chromatographic step into two fractions with sufficient resolution on the gas chromatographic column for the identification of seven pesticide residues from the adipose tissue sample and six pesticide residues from the liver tissue sample. These included: β- and γ-hexachlorocyclohexane (HCH), 1,4,5,6,7,8,8-heptachloro-2,3-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene (heptachlor epoxide), 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo,exo*-5,7-dimethanonaphthalene (dieldrin), 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene (*p,p'*-DDE), 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane (*p,p'*-DDD), and 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethane and 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2,2-trichloroethane (*p,p'*-DDT and *o,p'*-DDT, respectively). The concentration of the pesticides in tissue ranged from 0.073 to 28.7 ppm. Gas chromatographic separations were performed on a 4 ft × 1/8 inch o.d. coiled glass column packed with 3% OV-210 on 80/100 mesh Chromosorb W (HP). Figure 8 illustrates the total ion current chromatogram obtained by analysis of the adipose tissue extract representing 5 grams of tissue.

Instrumental sensitivity was sufficient to identify residues at concentrations of 0.30 ppm. Computer enhancement techniques (*21*) permitted identification of heptachlor epoxide residues at a level of 0.073 ppm.

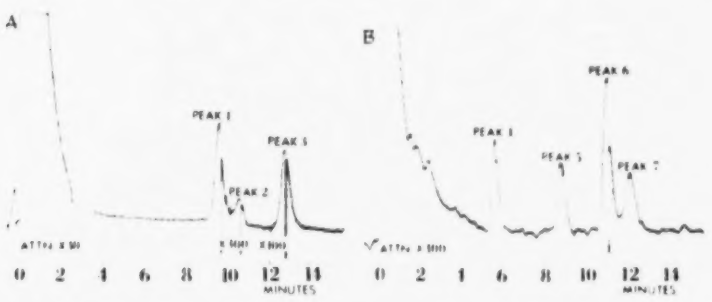

Figure 8. Total ion current chromatograms of extracts containing organochlorine pesticide residues isolated from adipose tissue (A, fraction 1 and B, fraction 2)

Programmed temperature conditions: one minute at 150° C to 200° C at 5° C/min, isothermal at 200° C

Definitive confirmation of pesticide residues was obtained by comparison of parent and fragment ion intensities and mass numbers of eluted pesticides and reference pesticides. Table I lists the residues encountered and the mass numbers and intensities of the characteristic fragments employed for identification in the adipose tissue sample. The mass spectral fragmentation patterns for all the compounds included in Table I with the exception of β-HCH have been adequately discussed by other investigators (*7*).

Table I. Gas Chromatographic Peak Identities and Characteristic Mass Spectral Fragments and Intensities of Some Organochlorine Pesticide Residues Isolated From Human Adipose Tissue

Peak No.	*Pesticide*	*Characteristic Mass Spectral Peaks and Intensities*
1	*p,p'*-DDE	m/e 246 (100%), 318 (83%), 316 (Molecular ion, 66%), 248 (58%), 320 (41%), 176 (41%), 210 (16%).
2	*o,p'*-DDT	m/e 235 (100%), 237 (70%), 165 (40%), 75 (20%), 199 (18%), 246 (18%), 352 (Molecular ion, 3%).
3	*p,p'*-DDT	m/e 235 (100%), 237 (72%), 165 (48%), 75 (22%), 50 (18%), 51 (9%), 352 (Molecular ion, 2%).
4	β-HCH	m/e 109 (100%), 219 (85%), 181 (78%), 183 (80%), 111 (62%), 193 (62%), 288 (Molecular ion, 4%).
5	Heptachlor epoxide	m/e 81 (100%), 353 (84%), 355 (76%), 351 (48%), 357 (35%), 237 (33%), 386 (Molecular ion, 8%).
6	Dieldrin	m/e 79 (100%), 108 (19%), 263 (18%), 277 (19%), 279 (16%), 345 (7%), 378 (Molecular ion, 3%).
7	*p,p'*-DDD	m/e 235 (100%), 237 (66%), 165 (58%), 75 (21%), 82 (16%), 88 (16%), 318 (Molecular ion, 5%).

Some difficulties were encountered in the absolute confirmation of configurational and structural isomers where mass spectral peak intensities and mass values of characteristic fragment ions were too similar under the conditions of the analysis. In those instances, however, identification was readily made on the basis of gas chromatographic retention time data. The estimated lower limit of detectability of organochlorine pesticide residues by this analytical scheme and instrument configuration was determined to be approximately 0.05 to 0.1 ppm.

Herbicide Residues

Combined methodology and metabolism studies involving the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) furnished an opportunity for the application of the gas chromatographic–mass spectrometric technique to the confirmation of derivatized intact residues and metabolites and the structural characterization of unknown metabolites isolated from rat urine. The analytical technique, briefly summarized, involves acid hydrolysis of phenolic conjugates, extraction of free phenols and acids with benzene, extract concentration and derivatization with diazoethane, and finally, column chromatographic cleanup and separation by silica gel chromatography employing benzene–hexane and benzene–ethyl acetate solvent systems as eluents (22). Programmed temperature gas chromatographic analyses of the urine extracts with a 7 ft × 1/8 inch o.d. aluminum column packed with 20% OV-101 on 60–80 mesh Gas Chrom Q provided the total ion current chromatograms shown in Figure 9. The mass spectral fragmentation data were used to confirm the presence of 2,4-D and 2,4,5-T as the ethyl esters and trichlorophenol (TCP) as the ethyl ether in the several

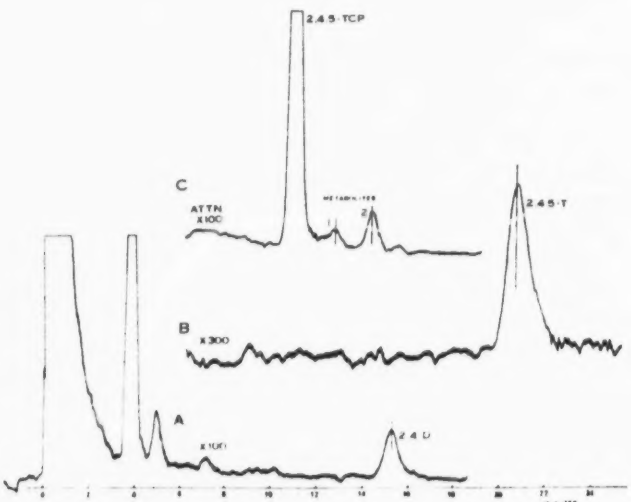

Figure 9. Total ion current chromatograms of animal urine extracts containing (A) 2,4-D, (B) 2,4,5-T, and (C) 2,4,5-trichlorophenol and an isomer of trichlorodihydroxybenzene, peak 2 (as ethyl esters and mono- and diethyl ethers, respectively)
Programmed temperature conditions: five minutes at 180 C to 220 C at 5 C/min, isothermal at 220 C

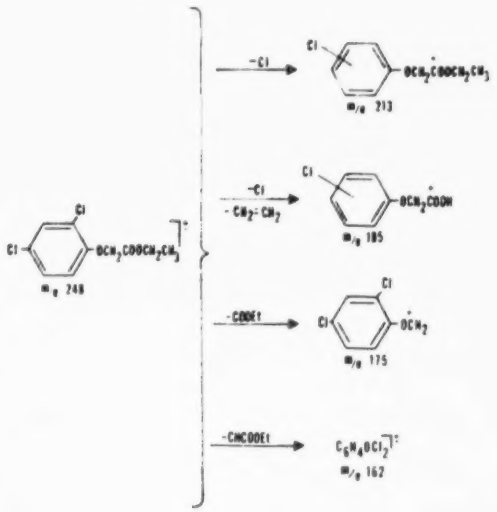

Figure 10. Electron impact-induced fragmentation scheme for 2,4-dichlorophenoxyacetic acid, ethyl ester

analyses performed. The diagnostic fragmentation pattern for 2,4-D ethyl ester included peaks at m/e 248, molecular ion; m/e 213 elimination of a Cl atom; m/e 185, consecutive elimination of a Cl atom and ethylene (C_2H_4) from the parent ion species; m/e 175, loss of the carboethoxyl function from the molecular ion, and finally, a peak at m/e 162 presumably arising from a dichlorophenol or dichlorocyclohexadienone-type radical ion (Figure 10). An analogous fragmentation scheme was observed for the ethyl ester of 2,4,5-T: m/e 282, molecular ion; m/e 247, loss of chlorine atom; m/e 219, consecutive elimination of a chlorine atom and a molecule of ethylene from the parent ion; m/e 209, decomposition of the parent ion with elimination of the carboethoxyl group, and finally, m/e 196, corresponding to a trichlorophenol or trichlorocyclohexadienone-type radical ion species.

In addition to the intact residues of 2,4,5-T excreted in the rat urine, mass spectral evidence was obtained for the presence of the metabolite TCP and a trichlorodihydroxybenzene isomer, which were observed as their mono- and diethyl ether derivatives, respectively. Thus, TCP ethyl ether displayed a molecular ion at m/e 224 and characteristic fragments at m/e 196 and m/e 160 owing to consecutive elimination of ethylene and HCl from the parent ion. The fragmentation pattern of 2,4,5-trichlorophenol was observed to exhibit similar behavior below m/e 196

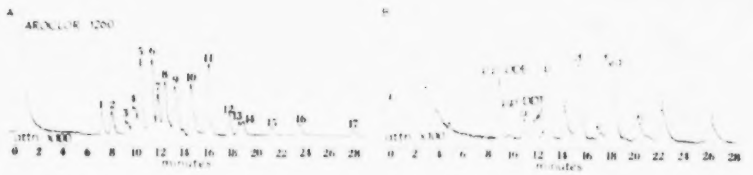

Figure 11. Total ion current chromatograms of (A) standard Aroclor 1260 mixture of polychlorinated biphenyls. Programmed temperature analysis: two minutes at 200 C, to 230 C min, isothermal at 230 C. (B) Human adipose tissue extract. Programmed temperature analysis: two minutes at 190 C, to 230 C at 5 C min, isothermal at 230 C

The mass spectral evidence for the trichlorodihydroxybenzene diethyl ether included a molecular ion of m/e 286 and significant fragment ions at m/e 240, m/e 212, and m/e 176 arising from the consecutive elimination of two molecules of ethylene and one molecule of HCl.

Polychlorinated Biphenyls

Confirmatory evidence for the presence of polychlorinated biphenyls (PCB) in fish, seabirds, conifer needles, and human depot fat has been obtained by gas chromatography–mass spectrometry (*23, 24*). The mass spectral behavior of standards of PCB materials (Aroclor 1254 and 1260) and PCB compounds isolated from human adipose tissue utilizing standard analytical methodology (*19*) has been reported recently (*25*). An illustration of the total ion current chromatogram obtained by programmed temperature analysis of a mixture of standard PCB compounds (Aroclor 1260) on a 100 ft × 0.02 inch i.d. stainless steel capillary column coated with OV-1 silicone oil is depicted in Figure 11. Most components of the Aroclor standard produced mass spectra with molecular ion groups of high intensity. In addition, the characteristic isotopic distribution patterns corresponding to the number of chlorine atoms in the parent ion and chlorine-containing fragment ions were observed. Other noteworthy features of the mass spectra were the relatively intense ion fragments resulting from consecutive loss of chlorine atoms from the parent ion and the presence of intense doubly charged fragments within the mass spectra of most of the PCB compounds. Thus, it was possible to establish the molecular weight and number of chlorine atoms associated with each major numbered component in the chromatogram of Figure 11. The standard Aroclor 1260 on this basis was shown to consist of at least two pentachlorobiphenyls, four hexachlorobiphenyls, six heptachlorobiphenyls, two octachlorobiphenyls, two nonachlorobiphenyls, and decachlorobiphenyl.

Although it was apparent that several of the major peaks in the chromatogram of the standard PCB material were in fact composed of more than a single component, no further attempt was made to elaborate on the identity or nonidentity of the individual components of each of these peaks.

Structural studies of the PCB components must necessarily involve the application of complex separation procedures coupled with detailed spectrometric studies of each isolated component. Figure 11 also includes the total ion current chromatogram of a human adipose tissue extract which was shown to contain traces of PCB compounds (peaks *a* through *j*) whose mass spectra were consistent with those found for Aroclor 1260, namely, peaks 6 and 9 through 17.

PCB materials in field-collected bald eagle specimens have been identified utilizing combined gas chromatography–mass spectrometry techniques in conjunction with thin-layer chromatographic separations of standard materials as well as tissue extracts (*26*). A total of 19 PCB compounds were detected in the field samples. Analyses were performed employing a spiral glass gas chromatographic column, 9 ft × 0.25 inch o.d., packed with 1% SE-30 on 100–120 mesh Gas Chrom Q. Mass spectra of the major components of Aroclor 1254 PCB standard were presented in this report as well as relative retention data for the individual components.

Discussion

Of all the systems which have been utilized for the analysis of pesticide residues, combined gas chromatography–mass spectrometry affords a particularly useful approach because positive identification of the components of a mixture can be made without prior separation at sensitivities compatible with the limited quantities of residues generally available. From the results of recent studies involving the application of this technique, it has been demonstrated that available residue analytical methods provide efficient isolation and adequate cleanup of extracts of human, animal, and environmental media in most cases to permit gas chromatographic–mass spectrometric analyses with maximum confidence. Additionally, it has been shown that this combined technique will conveniently provide definitive and conclusive confirmation of residue identity as well as characterization of residues and their metabolites of unknown structure.

In addition to the applications reported here, gas chromatography–mass spectrometry has been employed in the characterization of photochemical degradation products of *p,p'*-DDT and *p,p'* DDE (*27*), synthetic intermediates in *p,p'* DDT metabolism studies (*28*), and transformation products of herbicidal chloroanilines in soil (*29*).

From a technique standpoint, additional studies should be made on improvements of instrumental sensitivity to allow routine confirmation of pesticide residues at the nanogram and possibly the picogram level. Some gains have already been reported in this area, utilizing computer enhancement techniques (*21*), pulse height analysis (*30, 31*), and other methods (*4*). Studies documenting compound yield and enrichment factors for the several classes of organic pesticides and various gas chromatographic–mass spectrometric interfacial systems under a variety of operating conditions are required. Technological studies systematically designed to exploit the advantages of capillary and other gas chromatographic columns for pesticide residue analysis are also needed. Finally, development of a complete residue analysis system employing either an electron capture or flame ionization detector and splitter arrangement in tandem with the molecular separator permitting simultaneous mass spectrometric identification and detection and quantitation of pesticide residues from a single gas chromatographic injection would offer obvious advantages.

The reported applications of combined gas chromatography–mass spectrometry to the analysis of pesticide residues have been increasing in frequency in recent years. With developments in instrument technology and availability of instrumentation, it can be expected that the technique will be relied upon to an even greater extent in the future.

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RECEIVED September 3, 1970.

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PB 275 986
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INDUSTRIAL CHEMICALS

Sensitive Trapping and Gas Chromatographic Method for Vinyl Chloride in Air Samples

Environmental Protection Agency

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A simple and sensitive method is described for determining vinyl chloride monomer in air. Vinyl chloride monomer from a sample of air is trapped onto a Tenax GC trap cooled with Dry Ice. The trap is installed on a gas chromatograph by a simple valve arrangement. The trap is heated, and the entire sample is flushed onto the gas chromatographic column. Vinyl chloride is determined at 6 and 60 ppb (v/v) levels with recoveries ranging from 79 to 104%.

Vinyl chloride monomer (VCM) is a widely used industrial chemical which has recently been subjected to public controversy. The controversy results from evaluation of various scientific findings which implicate VCM as a health hazard under certain conditions and levels of exposure.

This pesticide laboratory became involved in VCM analytical methodology as a result of the use of VCM as a propellant in pesticide aerosols. The area of major interest was analyzing the VCM resulting from aerosol spraying in a room-air environment.

Numerous methods have been used for VCM analysis (1-3). Gas-liquid chromatography (GLC) appears to be a preferred method because of its sensitivity and its suitability for qualitative and quantitative analyses. However, under ambient conditions, at least 1-2 ppm (v/v) VCM must be present for analysis by direct syringe injection of an aliquot of a simple grab sample of air. If lower levels are to be analyzed, it is necessary to concentrate VCM prior to analysis. Such a concentration scheme might consist of a cold trap, trapping in a solvent, or trapping on an adsorbent from a measured quantity of air. If an adsorbent is used, VCM must be desorbed with a suitable solvent (4) or with heat as used by Zlatkis *et al.* (5-7).

Our technique was to adsorb VCM from an air sampling tube onto Tenax GC and heat desorb it directly onto the GLC column for measurement at ppb levels. Standards of VCM were injected into the air sample in the sampling tube to give a desired ppb concentration. The valve arrangement used for direct GLC adaptation is similar to that of Shields and Desai (8).

Experimental

Reagents

(a) *Vinyl chloride.*—Lecture bottle (Matheson Gas Products, 932 Patterson Plank Rd., East Rutherford, NJ 07073). Dilute as necessary.

(b) *Chromatographic support materials.*—(*1*) *Porapak® QS.*—80–100 mesh (Waters Associates Inc., Maple St., Milford, MA 01757). (*2*) *Tenax® GC.*—80–100 mesh (Enka N. V., Arnheim/Holland, supplied by Applied Science Laboratories, Inc., P.O. Box 440, State College, PA 16801). (*3*) *Gas-Chrom® S.*—45–60 mesh (Applied Science Laboratories, Inc.).

Apparatus

(a) *Gas chromatograph.*—Packard Model 802 equipped with flame ionization detector operated at 250 v, 3×10^{-11} and 3×10^{-12} amp full scale; gas flows (ml/min)—air 400, hydrogen 50, nitrogen carrier 40. Chart, 5 min/inch; column, 6′ (180 cm) × 4 mm id glass, packed with Porapak QS and operated at 120°C.

(b) *Gas sampling tubes.*—500 ml (No. 963504, Lawshe Instrument Co., Inc., 4926 St. Elmo Ave., Bethesda, MD 20014); 250 and 125 ml (No. K-653100, Kontes Glass Co., Vineland, NJ 08360). Exact volume of each tube was determined by water volume capacity.

(c) *Disposable pipets.*—145 × 6 mm id (A. H. Thomas Co., Philadelphia, PA 19105).

(d) *Gas-Chrom S trap.*—Prepare disposable pipet by centering 5 cm Gas-Chrom S with glass wool plugs.

(e) *Tenax GC trap.*—Construct 13.5 × 0.5 cm id trap as follows: Cut 13.5 cm portion from center of 5 ml graduated pipet and fire-polish ends. Center 6.5 cm Tenax GC within this tube with glass wool plugs. Fit bottom end with through-hole septum; fit top with dual, through-hole-puncture septum. Condition Tenax GC trap before use with nitrogen purge, 50 ml/min, 2 hr at 200°C.

(f) *Valves.*—Available from Lawshe Instrument

Co., Valve A, Hamilton 1LL1 (2-way); Valve B, Hamilton 3LLL3 (3-way).

(g) *Tenax GC trap heater.*—Wrap 10 cm length of copper tubing, 0.5" id (1.2 cm), with heating tape and insulate with asbestos tape and aluminum foil.

(h) *Septa.*—Dual through-hole-puncture, supplied by Packard Instrument Co., Inc., 2200 Warrenville Rd., Downers Grove, IL 60515.

Procedure

Assemble apparatus as follows (*see* Fig. 1): Fill sample tube by drawing air samples with vacuum line or pump. Close both sample tube stopcocks. Disconnect vacuum source. Connect compressed nitrogen gas to inlet of sample tube (579 ml in this case); keep stopcocks closed. Connect exit end of sample tube with short length of rubber tubing to Gas-Chrom S trap and this to Tenax GC trap, using dual through-hole-puncture septum. Connect exit of Tenax GC trap by means of through-hole septum to bubble meter. Cool both traps with finely crushed Dry Ice held by means of powder funnels fitted with punctured serum caps as shown in Fig. 1. Wrap funnels with towels to maintain cold.

Activate compressed nitrogen source, open both sample tube stopcocks, and collect for 35 min with nitrogen flow of 85 ml/min (measure by bubble meter). Discontinue nitrogen flow, remove Tenax GC trap, and quickly replace bottom through-hole septum with puncture septum. Immediately insert trap into GC system using valve arrangement shown in Fig. 2. Insert 0.03" (0.075 cm) id stainless steel tubing from valve B into dual puncture-through-hole septum in top of Tenax GC trap. Insert hypodermic needle from valve A into bottom puncture septum. Valve A should be in closed position and valve B should be in open position to route carrier gas only through side-arm of GLC column.

Preheat Tenax GC trap heater in positive position *above* Tenax GC trap. Lower heater over trap (*see* Fig. 2) and heat 5 min at 150–180°C (use thermocouple leads and pyrometer). After 5 min heating, turn valves A and B *simultaneously* to direct entire carrier gas flow through heated Tenax GC trap onto GLC column. After chromatographing sample, inject standards through septum in top of heated Tenax GC trap for comparisons and calculation. To remove trap, simultaneously close valve A and turn valve B to direct carrier gas flow to column side-arm.

Results and Discussion

Figure 3 is a chromatogram representing 579 ml air (collected from outside the laboratory window) spiked at 60 ppb VCM (v/v). A blank and standard are shown for comparison. Figure 4 is the same but spiked at the 6 ppb level. A 6 ppb blank and a standard are also shown. Collection efficiency has averaged about 90% at 6 ppb and 100% for 60 ppb. Table 1 lists recoveries at these 2 levels.

There are several advantages to this method. First, only a small air sample is required for analysis, thereby minimizing problems associated with techniques which must use long collection times and large volumes of air. Second, the maximum useful sensitivity of the GLC is realized. By injecting the entire sample, full use is made of the sensitivity available as contrasted to systems where VCM is desorbed with a solvent. In these latter cases only a small aliquot of sample

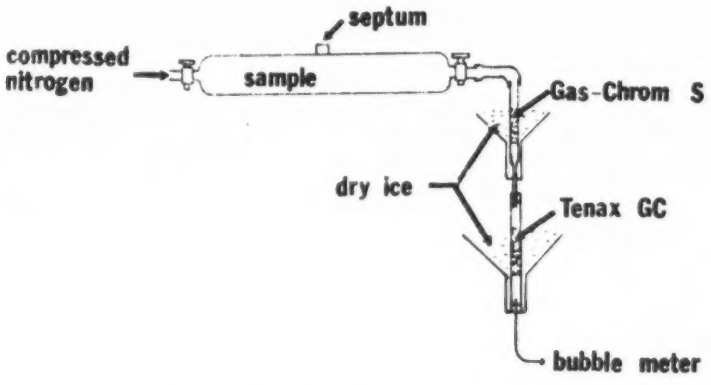

FIG. 1—Vinyl chloride trapping apparatus.

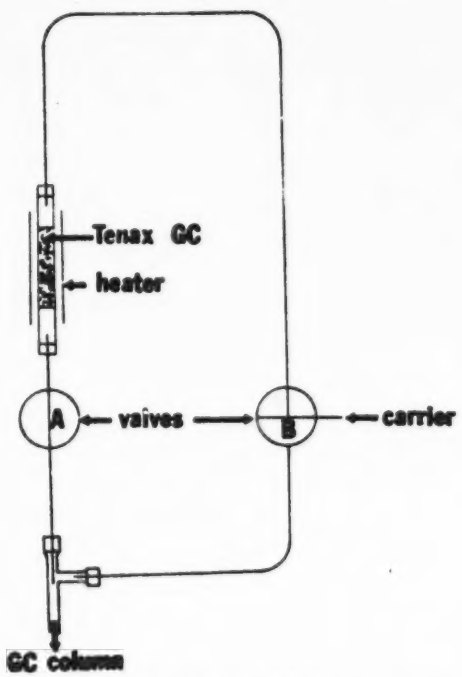

FIG. 2—Vinyl chloride trap installation to gas chromatograph.

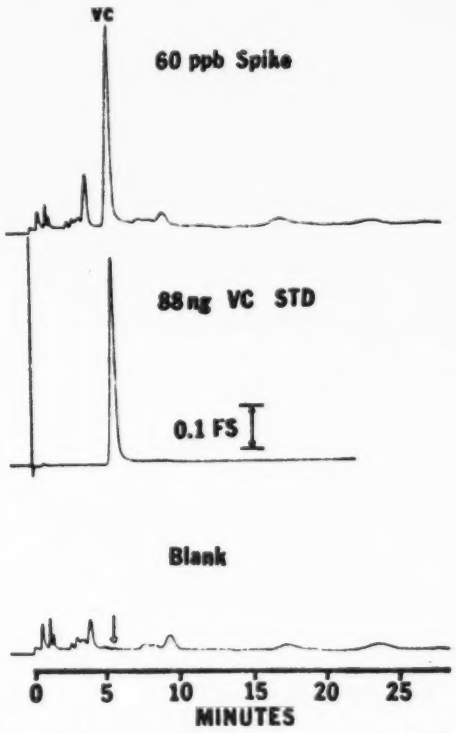

FIG. 3—Vinyl chloride in air analysis at 60 ppb, 3×10^{-11} amp full scale.

can be injected. There is also danger of interferences from solvent impurities.

One disadvantage of this collection approach is the need for cooling. The trapping efficiency is inadequate without cooling. Ice is not sufficient for this cooling; solid CO_2 (Dry Ice) is required. Water could be a problem but is essentially eliminated with the cold Gas-Chrom S trap and the use of only a small air sample. Water blockage problems can develop when large volumes of air are used (more than 30 L), although the Tenax GC will trap fairly efficiently even with this volume. Both traps may be used repeatedly. The water trap is regenerated by heating with a gas purge.

As with any analytical method, analyses by this approach should be confirmed by other techniques. Background responses vary considerably from day to day. Laboratory air gave considerable background responses because of fresh paint in the area. In one instance during these investigations, a response was observed at the retention time of VCM. This response was also observed in a second air sample collected at the same time when a solvent desorption technique (4) was used. Its appearance was short-lived, preventing determination of its source or identity. Use of more than one GLC column packing type or selective detectors such as micro coulometric may be advantageous in these instances.

Analyses of VCM at 6 ppb levels by using the trapping-concentration techniques suggest that, in theory, even lower levels could be detected, background interferences permitting. This method is a significant improvement in sensitivity over procedures currently in use. It also has advantages over concentration techniques using solvent desorption because aliquoting results in underutilization of the sensitivity of GLC. The GLC system used here does resolve VCM from Freons 11 and 12 in pesticide aerosols.

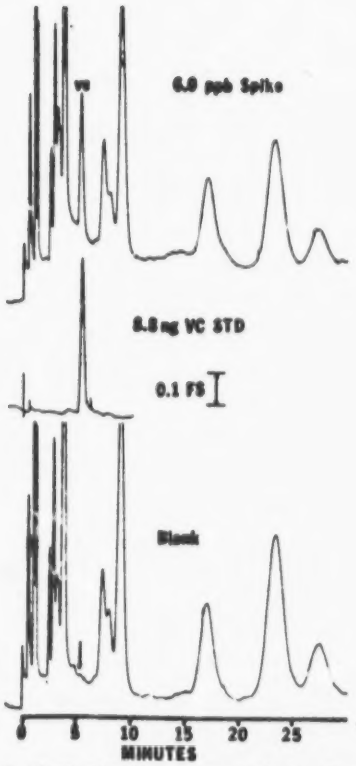

FIG. 4—Vinyl chloride in air analysis at 6 ppb, 3×10^{-13} amp full scale.

The techniques for using the total collected sample might also be used for VCM analyses of samples other than air.

Table 1. Per cent recoveries of VCM from air at 2 levels

6 ppb			60 ppb
80	83	90	98
90	97	100	98
91	81	97	104
95	85		
97	79		
Av. 89.6			Av. 100.0

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Received September 26, 1974.
This paper was presented at the 88th Annual Meeting of the AOAC, Oct. 14–17, 1974, at Washington, DC.

PB 275 987

A NOTE ON POLYCHLORINATED BIPHENYLS IN AIR

Frederick W. Kutz, Ph.D.* and Henry S. C. Yang

Abstract

Samples of ambient air were collected using an ethylene-glycol impinger sampler, and analyzed for selected pesticides and polychlorinated biphenyls in suburban locations in Florida, Mississippi, and Colorado. Preliminary results for samples taken in April, May, and June of 1975 show that PCB's were present at all locations.

Samples of ambient air were collected and analyzed for selected pesticides and polychlorinated biphenyls. Suburban locations in Miami, Florida, Jackson, Mississippi, and Fort Collins, Colorado, were sampled using an ethylene-glycol impinger sampler essentially the same as reported by Enos et al. (ref. 1) except that an improvement of air flow rate was made. The air flow rate was maximized and calibrated at ca. 30 l/min. Each sampler was operated for a 24-hour period to collect one sample. The analyses of samples were done by extraction with hexane followed by cleanup with fluorsil column chromatography, using a mixture of diethyl ether and petroleum ether for elution; the fraction containing the polychlorinated biphenyls and some organochlorine pesticides underwent further separation by silicic acid column chromatography. Quantitation of the polychlorinated biphenyls was done by summation of major peaks employing the dual-column gas chromatography with electron capture detector.

Preliminary results for samples taken in April, May, and June of 1975 show that PCB's were present at all locations. Although the data varied, the average concentration at each of the three locations was approximately 100 nanograms per cubic meter. The presence of polychlorinated biphenyls in ambient air samples taken in Miami and Jackson has been confirmed by combined gas chromatography-mass spectrometry. Initial identification of the polychlorinated biphenyls indicates that they were most comparable to the Aroclor 1254 standard.

In July the number of sampling sites was expanded to five with the addition of Lafayette, Indiana, and Harrisburg, Pennsylvania. Results from all sites should be available in the near future.

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PB 275 988

PESTICIDE PUBLICATION

Environmental Protection Agency
Chamblee, Georgia 30341

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

The National Human Monitoring Program for Pesticides

Anne R. Yobs[1]

The purpose of the human monitoring program is to determine on a national scale levels of pesticide incidence in the general population and to assess changes in these levels. Such incidence reflects prior exposure from all sources and is important in understanding the ecological impact of pesticides pollution and in studying the human health effects of pesticides exposure. Man may be exposed to pesticides through contact with any of the elements of the environment, including air, water, food, soil, and house dust, as well as in the course of some occupations and hobbies.

Exact measurement of man's total exposure to pesticides requires careful development and implementation of plans, the full cooperation of willing subjects, and adequate laboratory support—conditions which can be attained only in the controlled research situation and are not easily applicable to large groups. Previous human exposure to pesticides may be estimated from measurement of storage levels or excretion of these materials or their metabolites and from measurement of physiologic effects. No one of these approaches can be used to assess exposure to all pesticide chemicals. Some available methodologies are suited to research projects but not to field surveys involving large numbers of samples. Most studies reported in the literature have concerned acute pesticide poisoning or occupational exposure to pesticides, both situations in which rather massive exposure may occur. There are fewer published reports about the general population; these usually concern levels of chlorinated hydrocarbons in small groups from limited geographic areas.

In 1967, the Division of Pesticide Community Studies (formerly the Pesticides Program, CDC, PHS, DHEW) initiated a human monitoring program to determine levels of pesticide incidence in the general population of the Nation (*1*). Sensitivity limitations of available analytical methodology restricted this program to the measurement of certain chlorinated hydrocarbons in adipose tissue, specifically DDT and metabolites, dieldrin, heptachlor epoxide, and the isomers of BHC. Initially, it was necessary to resolve such administrative problems as sustained participation of pathologists, sample collection and handling, and data management, as well as problems of laboratory support including standardization of analytical procedures and laboratory comparability.

Pathologists were recruited at random in selected States of the contiguous 48 to collect small samples of adipose tissue from postmortem examinations and from specimens previously removed during therapeutic surgery. Samples were collected without fixative and stored at −4° C; specimens were shipped packed in dry ice. The limited information requested for each tissue donor included age, sex, race, and clinical pathology diagnoses. Geographic area of residence was presumed from the hospital's location, although it was recognized that some hospitals receive patients from a wide area of referral.

All analyses were performed by contract laboratories using only methodologies specified by the Division. These laboratories were equipped with gas-liquid chromatographs (MicroTek model 220) with electron capture detectors. All laboratories were required to maintain acceptable performance levels in the interlaboratory quality control program established and moderated for laboratories under contract to the Division by the EPA Perrine Primate Research Laboratory (formerly the Perrine Pesticide Research Laboratory). The Perrine facility also served as technical consultants for the analytical portion of this study.

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Evaluation of preliminary data from this study suggested that storage levels may vary in association with such factors as age, sex, race, and geographic location, and indicated that a statistical design was needed. (Data for the years 1967, 1968, and 1969 are now being prepared for publication.) This occurred at about the time the National Pesticide Monitoring Program was being reviewed by the Monitoring Panel of the Working Group on Pesticides, which is responsible to the Council on Environmental Quality.

While need for a statistical design for at least part of the sample collection was recognized, it was desirable to continue the operating procedure already established and with it tissue collection through pathologist and medical examiner cooperation.

The sampling population was therefore drawn from sources in cities of 25,000 or more population as shown in the 1960 census (2). (It was recognized that rural inhabitants are referred to nearby hospitals when such care is needed.) The contiguous 48 States were divided into the four census regions (fig. 1) with population distribution as follows: Northeast 28%, North Central 30%, South 24%, and West 17%. Based on anticipated laboratory performance, plans were made to collect 1950 samples of adipose according to the random statistical design. Samples would continue to be analyzed by contract laboratories following a rigid system of inter- and intra-laboratory control. Based on the estimate of 50 as the minimum number of specimens which could be collected from a given collection point annually with sustained interest and cooperation, the number of collecting points (or pathologists) was determined to be 39. These were allotted to each of the regions on the basis of population distribution: Northeast 11, North Central 12, South 9, and West 7.

FIGURE 1.—*United States census regions, 1960.*

Cities in each region were selected according to a random design, and provisions were made for the selection of alternate cities in those cases in which it proved to be impossible to secure samples. Cities of appropriate size were listed alphabetically by State and numbered consecutively within each State. Then they were ordered randomly using a table of random numbers. A running total of population was kept, and the sampling interval N was determined by dividing the number of pathologists per region into the total population of that region. A random number, M, between 1 and N was selected and the city in which that random number fell in the listing of running population totals was the first sampling site. Other sampling sites were located on this listing by adding $N + M$, $N + 2M$, etc. until the desired number of cities had been selected in each region. First alternate city was the next one on the list; second alternate the one immediately preceding the selected city. This series of alternatives has been sufficient. Cities initially selected by statistical design for tissue collection sites are listed below:

Northeast	North Central
Bridgeport, Conn.	Wichita, Kans.
Fitchburg, Mass.	Detroit, Mich.
Newburgh, N. Y.[1]	St. Joseph, Mo.
New York City, N. Y. (3)	Des Moines, Iowa
Ridgewood, N. J.	Oak Lawn, Ill.
Philadelphia, Pa.	Chicago, Ill. (2)
Bangor, Maine[1]	Salina, Kans.
Pittsburgh, Pa.	St. Louis, Mo.
New Rochelle, N. Y.	Belleville, Ill.
	Minneapolis, Minn.
	Lorain, Ohio
South	**West**
Houston, Tex.[1]	San Francisco, Calif.
New Orleans, La.	Tucson, Ariz.
Miami Beach, Fla.	Portland, Oreg.
Atlanta, Ga.	Gardena, Calif.[1]
Oklahoma City, Okla.	San Bernardino, Calif.[1]
Rock Hill, S. C.	Los Angeles, Calif.
Owensboro, Ky.	San Diego, Calif.
Macon, Ga.	
Washington, D. C.	

[1] Cities replaced by alternates during the first year of operation of the statistical design.
[2] Number of pathologists selected in city; where no number is given, assume the number to be one.

This design provided for sampling which would yield valid data concerning pesticide incidence variation with geographic distribution and with age, sex, and race variation of the donors. For each collecting point an annual sample quota was established further broken into three groups by age and sex: 14 years and under, male and female; 15 through 49 years, male and female; and 50 years and over, male and female. The number in each group was allocated proportionately according to the national age and sex distribution in 1960.

Sampling of nonwhites was limited to approximately 10% of the total samples collected. Stratification on a socioeconomic basis was considered but is not feasible at this time. Adipose tissue was to be collected from

postmortem and surgical specimens, on a quarterly basis, without regard for the donor's stated occupation. Since the program objective was to reflect the incidences in the normal (man-on-the-street) general population, samples were excluded from cases of known or suspected acute pesticide poisoning or chronic debilitating illness and from patients who had been institutionalized for long periods.

Experience with the age-sex quotas resulted in supplementing the original collection roster with pathologists at children's hospitals in order to adequately cover the youngest age groups and with active participants in the same or alternate cities to replace inactive participants.

Sample analysis was limited to determination of the same chlorinated hydrocarbons using a modified Mills-Olney-Gaither procedure (*3,4*) with thin layer chromatography for confirmation of results on every fifth sample. The percent lipid-extractable material was determined on each sample. Results were reported as parts per million on both wet-weight basis and lipid-extractable material basis. Data management procedures were unchanged.

Beyond the Minimum Program

It is impossible for the National Human Monitoring Program to answer questions about the health effects of pesticides by itself just as it is impossible to define pesticide incidence in a given locality by the statistical design discussed above, unless part of the samples happen to have been derived from that locality. Data and insights derived from the design are valuable as baselines and as background information for other studies.

To determine the health effects of pesticides exposure, the Division of Pesticide Community Studies established and maintains 14 epidemiology studies by contractual arrangement with State health departments and or local medical schools. The areas of these community epidemiology studies are listed below.

Arizona--Pima and Maricopa Counties
California—Imperial Valley
Colorado—Weld County
Florida—Dade County
Hawaii—Island of Oahu
Idaho—Ada County
Iowa—Johnson County
Michigan—Berrien County
Mississippi—Washington, Sunflower, and Bolivar Counties
New Jersey—Formulating Plants and Factories, entire State
South Carolina—Dorchester County
Texas—Hidalgo and Cameron Counties
Utah—Salt Lake County
Washington—Columbia River Basin, Yakima to Wenatchee

The emphasis in these projects is on prospective study of control and occupationally exposed populations in each study. Some tissues and samples of local environmental substrates are analyzed to determine local pesticide incidence. These projects are generally located in areas of heavy pesticide usage.

The Division also has technical assistance projects with other State health departments in the following 14 States to develop improved competence in the field of pesticides:

Arkansas	Montana	Oregon
Kansas	New Mexico	Pennsylvania
Kentucky	North Carolina	South Dakota
Louisiana	Ohio	Tennessee (Memphis-Shelby County)
Maine	Oklahoma	

As a part of these studies and to determine local pesticides incidence, samples of adipose are collected through the cooperation of selected local pathologists. These samples are handled as described above for samples in the National Pesticide Monitoring Program. Data from these samples are stored in the data bank with those from the statistical design. For some statistical testing these groups of data are combined, for others they are handled separately.

Future Program Modification

As refinement of methodology for the analysis of adipose samples permits, the human monitoring program will be expanded in scope to include additional pesticide chemicals or metabolites, as well as other pollutants such as the polychlorinated biphenyls (PCB's) which become identifiable. Already, oxichlordane, mirex, and the PCB's have been added. New methodologies have also been developed which permit estimate of human exposure to other groups of pesticides by measuring the excretion of parent compounds or metabolites in urine (*5*) (M. T. Shafik and H. F. Enos, *personal communication*). These are now being evaluated to determine if they can be used successfully in a survey monitoring program of the general population. The adoption of these methods will require modification of both sampling design and operating procedures and will present new problems in laboratory support. Changes in the program will be described when they are incorporated into the National Pesticide Monitoring Program.

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
from PESTICIDES MONITORING JOURNAL
Vol. 5, No. 1, June 1971

PESTICIDE
PUBLICATION

Environmental Protection Agency
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National Monitoring Program for Air

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Data on pesticides in air should be developed without special consideration for any one sector of the environment such as man, wildlife, etc. In order to do this the country should be divided on an arbitrary basis, i.e. along longitudinal and latitudinal lines or by some other grid mechanism constructed of x number of equally spaced lines east-west and y number of lines north-south across the country. Sampling should be conducted at approximately 60 sites selected by random design.

A rough design for air monitoring can still be devised; however, the limitations of the sampling equipment and analytical procedures selected for use will influence the length of sampling intervals and the time required for analysis of a sample. These factors will critically affect the number of samples which can be analyzed by a laboratory in a specific period of time.

Air should be sampled intermittently on a year round basis, at least three 24-hour samples to the month, with sampling equipment remaining in the same location for a minimum 12-month period. All sites should follow a uniform sampling schedule devised according to random design. Equipment should be located 15-40 feet above ground, and one location should be classified according to land usage although such classification should not influence site selection. Data on weather conditions in effect at the sampling location during each sampling period should be recorded including maximum-minimum temperatures, wind speed and direction, humidity, and precipitation. If equipment is limited, sample periods may be of 12 hours duration or less, but data should be compiled for 24-hour periods. All data should be reported as weight of pesticide per volume of air sampled.

For the foreseeable future, regardless of sampling technique, chemical analysis will be primarily by gas-liquid chromatography with appropriate detectors. Identification by GLC should be confirmed whenever amounts captured are sufficient to permit such procedures. In the case of heavy metals, especially mercury, flameless atomic absorption would be the method of choice.

The monitoring effort should include chlorinated hydrocarbons, organophosphates, phenoxyacetate derivatives, polychlorinated biphenyls, and certain other compounds that are widely used or are heavily used in restricted areas or on limited crops.

Such inclusion will, of course, increase the laboratory support required and consequently also increase budget requirements, especially when analytical procedures do not apply to more than one compound or group of compounds or require large amounts of time for completion.

Such a program will develop minimal data on pesticides in air which can be correlated with data from other parts of the National Pesticide Monitoring Program. It will also provide valuable background information for problem-oriented or other ambient air sampling.

In addition, provision must be made for research support for such an air monitoring program. This would include methodology development, equipment evaluation and development, as well as investigation of association of other parameters.

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